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# Antimicrobial effect of cetylpyridinium chloride against *Listeria monocytogenes* growth on the surface of raw and cooked shrimp

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**ANTIMICROBIAL EFFECT OF CETYLPYRIDINIUM CHLORIDE  
AGAINST *LISTERIA MONOCYTOGENES* GROWTH  
ON THE SURFACE OF  
RAW AND COOKED SHRIMP**

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Department of Food Science

by  
Tracie Michelle Dupard  
B.S., Xavier University of Louisiana, 2001  
August 2005

**Dedicated to My Parents,  
Patricia Dupard and the late Michael Dupard**

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## ABSTRACT

*Listeria monocytogenes* has emerged as a major foodborne pathogen for the seafood industry due to its psychrotrophic nature and its ubiquitous presence. It has been isolated from soil, sewage, dead vegetative matter, aquatic environments, fecal material, fish, crustaceans, and domesticated animals. As a result, *L. monocytogenes* has been responsible for several shrimp recalls and has been epidemiologically linked to human listeriosis.

Fresh seafood products are highly perishable and their shelf-life is limited by microbiological spoilage. Therefore, when pathogenic microorganisms are involved, it poses a health threat to the general public. The situation is further complicated because seafood processing plants are ideal environments for this organism to proliferate. As a result, this creates an ever growing potential for food safety issues.

Cetylpyridinium chloride (CPC) has been shown to have antimicrobial effects in decontaminating raw produce and poultry. Therefore, the objective of this study was to determine the effectiveness of cetylpyridinium chloride as a washing solution to inhibit *L. monocytogenes* growth on the surface of shrimp. Our studies have successfully shown the potential of cetylpyridinium chloride as a washing solution to reduce *L. monocytogenes* counts on the surface of raw and cooked shrimp stored at 4°C and -20°C. However, further investigations are necessary to determine its impact on sensory properties of shrimp as well as determining CPC residuals on the surface of raw and cooked shrimp. To date, the use of CPC has only been approved by the FDA at a level not to exceed 0.3 grams of CPC and should also contain propylene glycol at a concentration of 1.5 times that of the CPC per pound of raw poultry carcass.

## INTRODUCTION

An outbreak of listeriosis occurred in New Zealand (1980) which resulted in nine deaths that was epidemiologically linked to raw fish and shrimp (Lennon and others, 1984). Since this outbreak, the seafood industry has been concerned with the ability of *L. monocytogenes* to grow to high levels in shrimp when stored at refrigerated temperatures (Lennon and others, 1984; Mu and others, 1997; and Weagant and others, 1988). Most seafood products undergo a heat treatment at the consumer level prior to consumption, which reduces the probability of a foodborne outbreak. However some products may not receive this additional heating.

The consumption of seafood among American consumers has steadily increased over the past decade due to changes in lifestyles, stimulating the return of reduced fat and reduced calorie diets, and the greater awareness of health attributes of seafood, such as omega-3s. The U.S. Department of Agriculture projects that seafood and fish will increase 26% in per capita consumption between 2000 and 2020 (Sloan 2005). Recently, the National Restaurant Association reported that fish/seafood topped the list of menu items that consumers ordered more frequently compared to 2 years ago. America's favorite seafood, which is shrimp, rose 40% in chain menu entrees over the past five years.

However, fresh seafood products are highly perishable and their shelf-life is limited by microbiological spoilage (Mu and others, 1997). In addition, when pathogenic microorganisms are involved, it poses a health threat to the general public. The situation is further complicated when *Listeria monocytogenes* is involved since seafood processing plants are ideal environments for this organism to proliferate. As a result, this creates an ever growing potential for food safety issues. Therefore, the aim of my research is to determine the effectiveness of cetylpyridinium

chloride as a washing solution to eliminate or reduce *Listeria monocytogenes* on the surface of raw and cooked shrimp.

## LITERATURE REVIEW

*Listeria monocytogenes* is currently one of the major foodborne pathogens of concern for the seafood industry. There are many species of *Listeria*, however *L. monocytogenes* is the only species that is pathogenic to humans. *Listeria monocytogenes* is a gram-positive, facultative anaerobic, nonsporeforming, motile rod, consisting of one to five flagella. It is also a psychrotroph, but its optimal growth is at temperatures of 35-37°C (95-98.6°F). *Listeria monocytogenes* has emerged as a foodborne pathogen because of its ubiquitous presence (Appendix 1). It has been isolated from soil, sewage, dead vegetative matter, aquatic environments, fecal material, fish, crustaceans, and domesticated animals (Farber and Peterkin, 1991; Thimothe and others, 2002).

Due to its biological characteristics, *L. monocytogenes* have been difficult to control in food products. This pathogen is resistant to high levels of salt, freezing, drying, pH levels of 4.1 and above, as well as refrigerated temperatures and heat (Ray, 2001). Some authors have suggested that *L. monocytogenes* is also sensitive to high-temperature short-time pasteurization temperatures of 71.7°C (161°F) for 15 seconds or 62.8°C (145°F) for 30 minutes (Ray 2001; Lovett and others, 1990). As a result, heat resistance has been a controversial topic in the area of commercial pasteurization (Donnelly, 1990). Furthermore, other authors have suggested that pasteurization cannot inactivate *L. monocytogenes* (Doyle and others, 1987; Donnelly and others, 1990).

Consumption of foods contaminated with this pathogen can result in listeriosis. Human listeriosis affects pregnant women, immunocompromised individuals such as those individuals with cancer, AIDS, and diabetics, the elderly, and sometimes, healthy individuals. Healthy individuals usually experience mild flu-like symptoms. Symptoms associated with mild

listeriosis include: chills, diarrhea, headache, abdominal pain and cramps, nausea, vomiting, and fatigue. However, for high risk individuals, serious illness may include: septicemia, meningitis, encephalitis, and may even lead to death. Pregnant women who have become infected may experience flu-like symptoms; in addition, abortion of the fetus may occur. Approximately 2,500 cases of human listeriosis occur annually in the United States with 500 of these cases resulting in death.

Seafood products that have contributed to human listeriosis outbreaks include cold-smoked trout and smoked mussels (Brett and others, 1998; Ericsson and others, 1997; Miettinen and others, 1998; and Tham and others, 2000). Other seafood products that have been shown to be contaminated with *L. monocytogenes* include cold-smoked salmon and crawfish (Dalgaard and Jorgensen, 1998; Thimothe and others, 2002). Besides the outbreaks that have been linked to a particular food, other sporadic listeriosis outbreaks have occurred in which one or more foods were linked epidemiologically (Elliot and Kvenburg, 2000). In fact, during 1989 in Connecticut, nine cases of human listeriosis were reported from the consumption of shrimp resulting in one death (Riedo and others, 1990). It was reported that 2 of the cases were perinatal and 7 were nonperinatal. There were no immunocompromised individuals affected. In addition, during the 1980 in Auckland, New Zealand, 29 cases of human listeriosis were epidemiologically linked from the consumption of shrimp and raw fish, which resulted in nine deaths. Of the 29 cases reported, 22 were perinatal infections and 7 were the elderly and those individuals with cancer (Lennon and others, 1984).

Currently, the United States maintains a policy of "zero-tolerance" for *L. monocytogenes* in ready-to-eat foods, which is enforced by the Food and Drug Administration and the U.S. Department of Agriculture. This means that the detection of any *L. monocytogenes* in a 25-gram

sample of a food renders the food adulterated. However, in July of 1987, FDA officials in Seattle, Washington detected *L. monocytogenes* in samples of imported frozen raw shrimp (Ryser, 1991). However, no recalls were issued, but this incidence prompted the FDA to initiate a survey (Anonymous, 1987). In this survey, the FDA officials at each district office were ordered to collect and test six imported frozen raw shrimp samples per month through their ports of entry using the original FDA procedure (Appendix 2) for isolating *Listeria*, with the samples representing as many different countries as possible. In addition, each district was also ordered to collect and test three domestic frozen raw shrimp samples at the wholesale and retail level per month for all species of *Listeria*. Headless, frozen raw shrimp samples were collected between July and October of 1987 from ten different countries and examined. Of these samples tested, *L. monocytogenes* was isolated from 4 of the 74 imported samples of frozen raw shrimp, with all the positive samples deriving from Central and South American countries. Consequently, the lots obtained from Honduras were found to contain  $10^4$  cells/g *L. monocytogenes* (McCarthy and other, 1990). In addition, the lots obtained from Ecuador were found to contain  $10^4$  cells/g of *L. innocua* in shell-on shrimp and  $10^5$  cells/g of *L. innocua* in peeled shrimp (McCarthy and other, 1990). However, since shrimp are not consumed in the raw state, no recalls were issued. Unfortunately, no reports have been issued for the domestic frozen raw shrimp samples.

In later years however, contamination of foods with *L. monocytogenes* have been responsible for several Class I recalls. According to 21 CFR 7.3(m)(l), a Class I recall is initiated when there is a reasonable probability that the use of, or exposure to, a violative product will cause serious adverse health consequences or death. Between 1987 and August 1998, there were seven Class I recalls in the U.S. for domestic or imported ready-to-eat shrimp products. The locations of these manufacturers were Florida, Georgia, Maine, New York, and Washington,

of which more than 31,332 pounds of shrimp were affected (Elliot and Kvenburg, 2000).

Poor sanitation of food contact surfaces, equipment, and processing environments has been a contributing factor to foodborne outbreaks and food recalls (Chmielewski and Frank, 2003). Some seafood environments *L. monocytogenes* have been isolated from include drains, floors, condensate lines, crates, door handles, and conveyor belts (Destro and others, 1996; Hoffman and others, 2003; Thimothe and others, 2002). Improperly cleaned surfaces can lead to the buildup of soil, which in the presence of water may result in the development of microbial biofilms. In addition, cross contamination may also occur when food is passed over these contaminated food contact surfaces.

Biofilm formation does not occur suddenly. Generally speaking, biofilms are a layer of bacteria that attach too surfaces and to one another with the help of polymeric materials anchored to a surface, which trap other bacteria, debris, and nutrients. As a result of this buildup, a microbial film, or biofilm, is established. Not only do biofilms provide protection to the microorganisms, they also provide the microorganisms with a source of food and nutrients, which in return allows the microorganisms within these biofilms to act synergistically as they are permitted to grow (Stier 2005). There are many types of microorganisms, both non-pathogenic and pathogenic, that can form biofilms. Non-pathogenic microorganisms include *Pseudomonas fragi*, *Enterococcus* spp., and *Pseudomonas fluorescens*, while pathogenic microorganisms include *Salmonella*, *E. coli O157:H7*, and *L. monocytogenes*.

The initial step in biofilm formation is the attachment of an organism to a surface. Attachment often occurs in two stages, reversible followed by irreversible adhesion (Chmielewski and Frank, 2003). As a result, the initial attachment is reversible and often occurs within five to thirty seconds. This attachment is very weak, involving only van der Waals forces,

electrostatic forces, and hydrophobic interactions. During reversible attachment, microorganisms still exhibit Brownian motion and are easily removed by the use of mild shear force (Chmielewski and Frank, 2003).

Irreversible attachment results from the anchoring of appendages (such as pili, flagella, or adhesion proteins) and the formation of polysaccharide-like material or polymers. These polysaccharides act as an adhesive allowing the cells to cement to the surface as well as to one another. Also, the polysaccharides assist in trapping other cells and debris. The bond between the microbial appendages and the surface usually occurs within twenty-four hours of contact and involves dipole-dipole interaction, hydrogen bonds, hydrophobic bonds, and ionic covalent bonds. During irreversible attachment, the removal of attached cells is difficult and requires the use of strong shear force through the application of detergents, sanitizers, heat, and surfactants (Chmielewski and Frank, 2003; McCarthy, 1992; Stopforth and others, 2002).

A mature biofilm is a system that reaches equilibrium. This means that the flowing product delivers all elements that are necessary for its growth and survival, as well as carries away debris that has been sloughed from the surface. Once equilibrium is reached, the film remains at a certain thickness and remains that way (Stier, 2005).

Not only do biofilms form on various environmental surface types, such as stainless steel, plastic, glass, copper, and rubber (Somers and Lee Wong, 2004; Stier, 2005; and Stopforth and others, 2002), some studies suggest that they may also form on food surfaces, such as chitin (McCarthy, 1992). Chitin is the main structural unit of the exoskeleton of crustaceans such as shrimp. It is a nitrogen-containing polysaccharide that provides strength and protection to the organism. According to McCarthy (1992), when chitin flakes were inoculated with a  $10^7$  *L. monocytogenes* cells/mL and incubated at 25°C for two days or seven days, electron micrographs



illustrated that a 2-day biofilm exhibited many single cells attached to the chitin flakes, but no confluent film. The presence of fimbriae was observed, indicating attachment to the surface. However, the more complex 7-day biofilm exhibited many more cells and fimbriae-type structures.

Therefore, since *L. monocytogenes* is associated with shrimp and can contaminate raw seafood as well as post-processed seafood, researchers are examining the effects of rinse treatments that will yield a reduction of *L. monocytogenes* on the surface shrimp (Mu and others, 1997; Wang and Johnson, 1997).

One approach to combat *L. monocytogenes* is the use of cetylpyridinium chloride, which is commonly referred to as CPC. It is currently used as an active antimicrobial ingredient in mouthwash and throat lozenges. Cetylpyridinium chloride is a cationic surfactant belonging to the group of quaternary ammonium compounds (QACs), which are the most useful antiseptics and disinfectants (McDonnell and Russell, 1999). These cationic surfactants are polar molecules with a positively charged head and a long, uncharged hydrocarbon chain. The head contains a central nitrogen nucleus with various alkyl groups (R) attached (Talaro and Talaro, 1993). Quaternary ammonium compounds are membrane active agents and are known to lower cellular surface tension, disrupt the bacterial cell membrane, and cause loss of selective permeability of the bacterial cell membrane (Talaro and Talaro, 1993). Salton (1968) proposed a sequence of events occur during the mechanism of action of QACs on bacteria: (1) adsorption and penetration of porous cell wall; (2) interaction with cytoplasmic membrane (lipid-protein) followed by membrane disorganization; (3) leakage of intracellular low molecular weight constituents, such as amino acids, nucleotides, ions; (4) degradation of proteins and nucleic acids; and (5) lysis due to wall-degrading autolytic enzymes (Appendix 3).

In relation to Gram-positive bacteria, such as *Listeria monocytogenes*, the cell membrane has a high affinity to QACs. Therefore, the membrane is readily dissociated. However, QACs activity may be greatly reduced by organic matter (Talaro, 1993). At medium concentrations, QACs are effective against Gram-positive microorganisms, viruses, fungi, and algae. At low concentrations, QACs may exhibit microbistatic effects, which mean that growth of microbes is inhibited without killing them. However, QACs are less effective on Gram-negative microorganisms than Gram-positive. The reason for this is because the outer membrane contributes an extra barrier in Gram-negative forms to slow or stop the entry of some antimicrobial agents (Talaro and Talaro, 1993). As a result, this makes the Gram-negative microorganism generally more difficult to destroy than Gram-positive microorganisms.

Cetylpyridinium chloride was developed by Safe Foods Corporation under the brand name "Cecure". In March 2004, a dramatic breakthrough in food safety occurred when a research team of scientists led by Danny Lattin, Ph.D., at the University of Arkansas for Medical Sciences (UAMS) in Little Rock, and Michael F. Slavik, Ph.D., at the University of Arkansas Poultry Science Center in Fayetteville, discovered that Cecure is extremely effective in killing most food-borne pathogens that cause serious and sometimes life-threatening illnesses, including *Listeria monocytogenes*, *E. coli*, *Salmonella*, and *Campylobacter*, resulting in a 3 to 6 Log reduction (University of Arkansas for Medical Sciences, 2004).

Cetylpyridinium chloride is a versatile ingredient with several application options, such as pre-chill, post-chill, and pre-package. It can also be used on ready-to-cook, ready-to-eat, and processed products manufactured from poultry, meat, and fish. Cetylpyridinium chloride is typically applied using a fine mist, spray, or a rinse. Some foods may even be dipped. To be treated, foods are usually passed through a spray cabinet, chamber, or a tunnel. According to

Safe Foods Corp., CPC shows no adverse organoleptic effects when it is applied properly. It does not impact flavor, texture, appearance, or the odor of foods. Its pH is near neutral, and it is stable, non-volatile, and soluble in water. It was noted by Bosilevac and colleagues (2004) that the allowable limit for an average adult (70kg or 154.3lbs. in body weight) has been determined to be 4.4 mg/day. In addition, according to Dr. Amy Waldroup, Senior Advisor, Food Safety for Safe Foods and former Professor of Poultry Science at the University of Arkansas at Fayetteville, “an individual would have to consume 90,000 pounds of Cecure-treated chicken per year to consume as much CPC as they would presently consume using a popular over-the-counter CPC-mouth rinse daily” (Safe Foods Corporation, 2003).

As of April 2, 2004, the Food and Drug Administration (FDA) has amended the food additive regulations to provide for the safe use of cetylpyridinium chloride as an antimicrobial agent in poultry processing. The FDA has regulated that CPC be used to treat the surface of raw poultry carcasses. The additive is applied as a fine mist spray of an ambient temperature aqueous solution to raw poultry carcasses as it passes down the production line prior to immersion in a chiller, at a level not to exceed 0.3 gram cetylpyridinium chloride per pound of raw poultry carcass. The aqueous solution should also contain propylene glycol at a concentration of 1.5 times that of the cetylpyridinium chloride. The overspray is captured and recycled in the process, leaving no environmental impact (FDA, 2004).

Cetylpyridinium chloride has also been shown to have antimicrobial effects in decontaminating raw beef, poultry, and produce (USDA, 2004; UAMS, 2004; Wang and Slavik, 2001). The use of cetylpyridinium chloride for removal of *L. monocytogenes* on fresh beef and fresh-cut vegetables were found to be concentration-dependent, with the most effective concentration being 0.5% CPC, yielding approximately a 3.25 and 3.70-log reduction,

respectively (Lim and Mustapha, 2004; and Wang and others, 2001).

Lim and Mustapha (2004) investigated the effect of 0.5% cetylpyridinium chloride (CPC) against *L. monocytogenes* inoculated on the surface of fresh beef during storage at 4°C for two weeks. Whole beef round was purchased from a local retail store and aseptically trimmed to reduce natural microflora. The trimmed beef was cut into cubes and exposed to ultraviolet light to further minimize natural surface microflora. The raw beef samples were then dip inoculated into a  $10^5$  to  $10^6$  CFU/g *L. monocytogenes* Scott A solution for one minute and allowed to drip dry for ten minutes. Following this inoculation, the raw beef samples were placed on absorbent pads that were previously sprayed with 20 ml of six antimicrobial formulations consisting of CPC, acidified sodium chlorite (ASC), and potassium sorbate (PS). Next, the samples were then aseptically packed in a commercial foam tray pack and wrapped with polyvinyl chloride film. The samples were stored in a commercial display refrigerator that was lit by a fluorescent lamp to stimulate grocery store conditions. This study concluded that surface sanitization using CPC, ASC, or an equal mix of these two agents effectively reduced microbial numbers on the beef during storage. Their results showed that 0.5% CPC had a 3.25-log reduction of *L. monocytogenes* counts, while the mixed solutions were not as effective as ASC or CPC alone (Lim and Mustapha, 2004).

In addition to these findings, Wang and colleagues (2001) established similar results for fresh-cut vegetables (broccoli, cauliflower, and radishes). The effect of 0.1% and 0.5% cetylpyridinium chloride (CPC) at decreasing the number of *L. monocytogenes*, *E.coli* O157:H7, and *Salmonella Typhimurium* counts on fresh-cut vegetables were investigated. Vegetables samples were purchased from a local retail store on the day of testing. The vegetables were cut into 25 g samples and dip inoculated into  $10^5$  CFU/ml culture solutions for one hour at room

temperature. After inoculation, all samples were rinsed with tap water for one minute. Next, the vegetables samples were treated with either a water control treatment, a 0.1% CPC treatment, or a 0.5% CPC treatment. The protocol for the CPC treatment stated that five samples of each vegetable were immersed into one liter of 0.1% or 0.5% CPC for one minute at room temperature. The control samples were treated the same, except distilled water was used instead of CPC. All samples were then rinsed with tap water for one minute, placed into individual sterile bags with 225 ml of 0.1% BPW, and shaken vigorously by hand for one minute. All samples were serially diluted, and 0.1 ml was surface plated on a selective medium. The experiment was replicated in triplicate and statistical analysis was performed using JMP-IN. Also, after treatments, the vegetables and their washing solutions were tested for CPC residuals by using high-performance liquid chromatography with an on-line dual  $\lambda$  absorbance detector.

It was observed that the effect of 0.5% CPC was significantly different ( $P < 0.05$ ) from the 0.1% CPC treatment on reduction of *L. monocytogenes*. *Listeria monocytogenes* counts were reduced by 2.85 and 3.70 log CFU/g when treated with 0.1 and 0.5% CPC, respectively, in comparison with the vegetables treated with water only (Wang and others, 2001). It was further observed that CPC-treated vegetables resulted in a greater reduction of *L. monocytogenes* and *S. Typhimurium* than *E. coli O157:H7*. In addition, results showed that the effect of CPC treatments on the reductions of attached bacteria to the vegetables surfaces varied, depending on the types of vegetables and microbial strains. For the CPC residual test, results showed that on 0.5% CPC-treated vegetables, the residues were very low ( $<23\mu\text{g/g}$ ). In all three vegetable washing solutions, the residues were undetectable.

This study concluded that CPC was effective in reducing *S. Typhimurium* on the surface of vegetables, but CPC was ineffective against *E. coli O157:H7*. However, it has been reported

that CPC is bactericidal to gram-positive bacteria, but less effective against gram-negative bacteria. This may have resulted from the differences in the type and contents of phospholipids between the two groups of organisms (Robinson, 1970).

Rodriquez-Morales and colleagues (2005) also studied the residual levels on CPC-treated apples using high-performance liquid chromatography because this method is specific, sensitive, reproducible, and accurate. The limit of quantification, which is defined as the lowest concentration that can be determined with acceptable precision and accuracy, was 1.0 µg/g. When this method was applied to evaluate residuals on five waxed Granny Smith apples (150 g) and five non-waxed Arkansas Black apples (150 g), it was observed that the Granny Smith apples treated with aqueous CPC solutions of 2 and 4 mg/ml exhibited 4.35 and 4.33 µg/g of CPC residue, respectively. For the Arkansas Black apples, the residue levels were 3.21 and 2.35 µg/g, respectively, when treated with 2 and 4 mg/ml solutions of CPC. These findings were even lower than those previously reported for vegetables (<23 µg/g) and beef (undetectable) (Wang and others, 2001; and Bosilevac and others, 2004).

Although these studies indicate the potential of cetylpyridinium chloride as a washing solution to eliminate or reduce *L. monocytogenes* from the surfaces of raw poultry, fresh-cut vegetables, and fresh beef, further investigation is needed to determine the effectiveness of the use of this antimicrobial agent for shrimp decontamination.

## MATERIALS AND METHODS

**Culture Preparation:** *Listeria monocytogenes* V7 (1/2a) was obtained from the United States Food and Drug Administration. The culture was maintained at a refrigerated temperature at 4°C on a Brain Heart Infusion slant. One loopful (10µl) of cells were transferred to 12 ml of Brain Heart Infusion (BHI) broth (Difco, Detroit, Mich.) and was incubated at 37°C for 16 h prior to use.

**Shrimp Samples:** For the purpose of the following studies, retail and domestic shrimp were used.

Previously frozen, raw white shrimp (with and without shell) and cooked shrimp were purchased from a local retail supermarket. Each shrimp package was labeled as farm-raised and imported from Indonesia. Also, the ingredients listed on each of these shrimp packages contained sodium tripolyphosphate.

One lot of wholesale, individually frozen domestic white shrimp that had not treated with sodium tripolyphosphate were also used. These shrimp samples were wild-caught from the Gulf of Mexico and were purchased with head intact.

**Microbial Inocula Preparation:** Each shrimp sample was weighed to approximately 5g. A loopful (10µl) of *L. monocytogenes* was transferred to 5 ml of BHI then grown at 37°C for 16h. The culture was then added to a sterile dip cup and diluted with 45 ml of PBS buffer. The shrimp samples were dipped into culture for 1 minute and then allowed to air dry for 1 h to ensure adhesiveness of cells to the sample surface. Following inoculation, each shrimp was placed in a sterile plastic bag and then treated with CPC as described below.

**CPC Preparation:** Seven aqueous solutions of cetylpyridinium chloride (CPC) were prepared in sterilized deionized water at concentration levels of 0.05%, 0.1%, 0.2%, 0.4%, 0.6%,

0.8%, or 1.0%. All solutions were made fresh prior to conducting experiment and were used at room temperature within 1 h.

**Study 1 - CPC Treatment of Retail White Shrimp:** Sixteen shrimp samples were selected from three groups of previously frozen retail shrimp: raw shrimp with and without shell or cooked shrimp. The shrimp were randomly selected, weighed to 5g, and divided into two groups. One shrimp sample from each group was used as the control. All 16 samples were dipped into PBS containing *L. monocytogenes* for 1 min then the shrimp samples were placed under a laminar flow hood for 1 hour to allow the bacteria to attach onto the shrimp surface. Shrimp samples were treated with CPC in 2 groups, one group with a water wash and the other without a water wash. Each shrimp sample was placed in a sterile Whirl-pak bag, treated with 50 ml of aqueous CPC solutions, and agitated for 1 min. Each shrimp from the first group was then placed into individual, clean, sterile Whirl-Pak bags in which 45 ml of PBS buffer solution was added, and allowed to homogenize in a stomacher for 1 min. Following the CPC treatment for the second group of shrimp, each shrimp was placed into individual, clean, sterile bags in which 50ml of sterile deionized water was added and agitated again for 1 min. The water was poured out of each bag and then 45 ml of PBS buffer solution was added, and allowed to homogenize in a stomacher for 1 min. The log CFU/g of *L. monocytogenes* was determined immediately after treatments as described above (Appendix 6).

**Study 2 – Attachment of *Listeria monocytogenes* 1/2a onto the Surface of Retail Raw and Cooked Shrimp:** Eight shrimp samples were selected from three groups of previously frozen retail shrimp: raw shrimp with and without shell or cooked shrimp. The shrimp were weighed to 5g. All 8 samples were dipped into PBS containing *L. monocytogenes* for 1 min then the shrimp samples were placed under a laminar flow hood for 1 hour to allow the bacteria



to attach onto the shrimp surface. Shrimp samples were placed into individual, clean, sterile Whirl-Pak bags in which 45 ml of PBS buffer solution was added, and allowed to homogenize in a stomacher at normal speed for 1 min. The log CFU/g of *L. monocytogenes* was determined immediately after inoculation as described above (Appendix 7).

### **Study 3 - Ninety-Day Shelf-Life Study on Domestic (Wild-Caught) White Shrimp:**

One hundred ninety-two white shrimp samples were randomly selected from the lot of domestic shrimp. The heads of the shrimp were removed and the shrimp were weighed to approximately 5g, and divided into three groups. Each of the three groups was used to conduct a 90-day shelf-life study at freezing temperature of -20°C. For the purpose of this study, concentrations of 0.05%, 0.4%, and 1.0% CPC were used and log CFU/g of *L. monocytogenes* was determined on day 0, 7, 14, 21, 30, 60, and 90. The first group was used as headless, shell-on, raw shrimp. The second group was hand peeled, while the third group was cooked with heads removed in sterile Whirl-Pak bags in a boiling water bath for 5 minutes. Two shrimp samples from each group were used as the control. All of the samples were dipped into PBS containing *L. monocytogenes* for 1 min then the shrimp were placed under a laminar flow hood for 1 hour to allow the bacteria to attach onto the shrimp surface.

Next, the three shrimp sample groups were then treated with CPC, with or without water wash. Each shrimp sample was placed in a sterile Whirl-Pak bag, treated with 50 ml of aqueous CPC solutions, and manually agitated with medium force for 1 min. Following the CPC treatment for the water rinsed group, each shrimp was placed into individual, clean, sterile Whirl-Pak bags in which 50 ml of sterile deionized water was added and then the samples were agitated again for 1 min. Each of the samples were then placed into labeled, individual, clean, sterile Whirl-Pak bags in which 45 ml of PBS buffer solution was added, and allowed to

homogenized in a stomacher at normal speed for 1 min. The log CFU/g of *L. monocytogenes* was determined on day 0, 7, 14, 21, 30, 60, and 90 (Appendix 10).

**Study 4 - Nine-Day Shelf-Life Study on Domestic White Shrimp:** Ninety-six white shrimp samples were randomly selected from the lot of domestic shrimp. The heads were removed and the shrimp were weighed to approximately 5g, and divided into three groups. Each of the three groups was used to conduct a 9-day shelf-life study at a refrigeration temperature of 4°C. For the purpose of this study, the same procedure was used as described in the previous 90-day shelf-life study except log CFU/g of *L. monocytogenes* was determined on day 0, 3, 6, and 9 (Appendix 8).

**Study 5 - Biofilm:** Sixty domestic white shrimp samples were randomly selected from the same lot of domestic shrimp. The shrimp were weighed to approximately 5g, and divided into three groups. Each of the three groups were used to conduct an 8-day biofilm study at refrigeration temperature of 4°C. For the purpose of this study, the log CFU/g of *L. monocytogenes* was determined at day 0, 2, 4, 6, and 8. The first group was used as headless, shell-on, raw shrimp, the second group was hand peeled, and the third group was cooked with heads removed in sterile Whirl-Pak bags in a boiling water bath for 5 minutes. All of the samples were dipped into PBS containing *L. monocytogenes* for 1 min then the shrimp were placed under a laminar flow hood for 1 hour to allow the bacteria to attach onto the shrimp surface. Each shrimp sample was then placed in an individual sterile Whirl-Pak bag labeled for day 2, 4, 6, or 8. On the day of analysis, each shrimp sample was treated with 50 ml of aqueous CPC solutions (0.05%, 0.4%, and 1.0%), and manually agitated with medium force for 1 min. Each of the samples were then placed into labeled, individual, clean, sterile Whirl-Pak bags in which 45 ml of PBS buffer solution was added, and homogenized in a stomacher at normal speed

for 1 min. The log CFU/g of *L. monocytogenes* was determined immediately after treatments as described above on day 0, 2, 4, 6, and 8 (Appendix 9).

**Enumeration of Pathogen:** To determine *L. monocytogenes* counts, 45 ml of PBS buffer was added to the shrimp samples and then homogenized in a stomacher for 1 minute at normal speed. Serial dilutions were prepared for each sample and 0.1mL portions of each dilution were surface plated onto Oxford medium with selective supplement SR140 (Oxoid LTD., Basingstoke, Hampshire, England). *Listeria monocytogenes* counts were determined on these plates following incubation of plates at 37°C for 48h.

**Statistical Analysis:** Reductions caused by CPC treatments with and without water rinses in *L. monocytogenes* counts on raw and cooked shrimp were analyzed by statistical comparisons of all pairs using one-way analysis of the variance using ANOVA procedures of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA). Means and standard deviations were determined by student's t-test. The statistical difference was set at  $p > 0.05$ . All experiments were repeated in duplicate.

## RESULTS

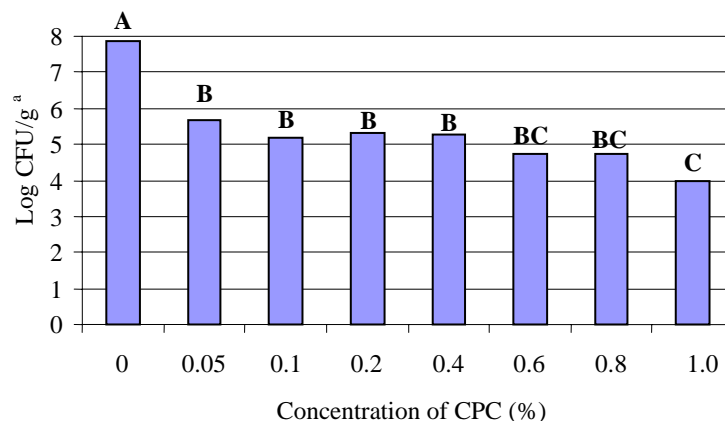


Figure 1: The effect of cetylpyridinium chloride against *L. monocytogenes* 1/2a on the surface of retail headless, shell-on, raw shrimp without a water rinse stored at 4°C for 24h.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

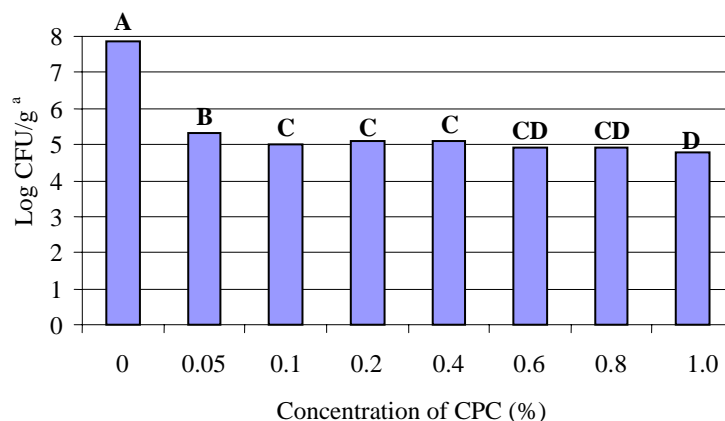


Figure 2: The effect of cetylpyridinium chloride against *L. monocytogenes* 1/2a on the surface of retail headless, shell-on, raw shrimp with a water rinse stored at 4°C for 24 h.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

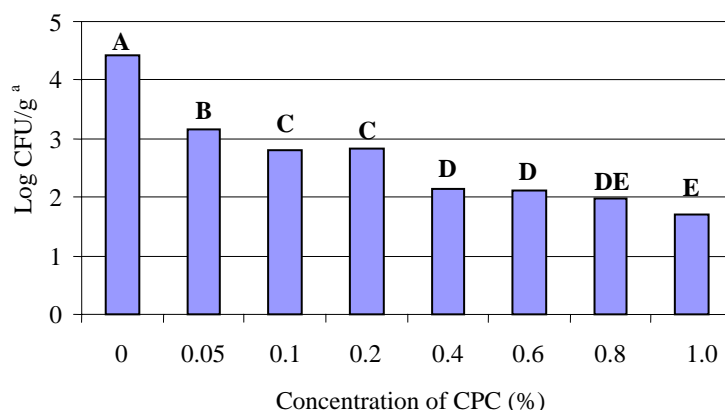


Figure 3: The effect of cetylpyridinium chloride against *L. monocytogenes* 1/2a on the surface of retail peeled, raw shrimp without a water rinse stored at 4°C for 24 h.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

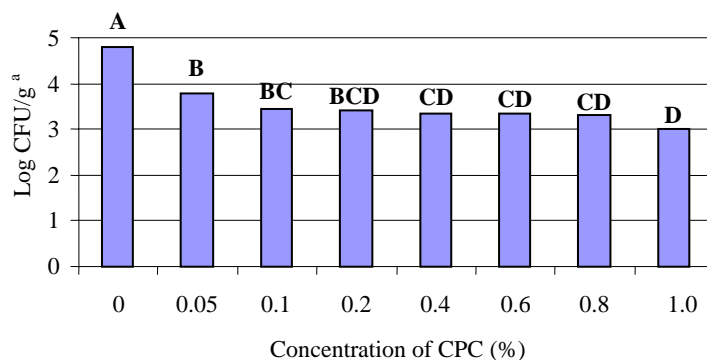


Figure 4: The effect of cetylpyridinium chloride against *L. monocytogenes* 1/2a on the surface of retail peeled, raw shrimp with a water rinse stored at 4°C for 24 h.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

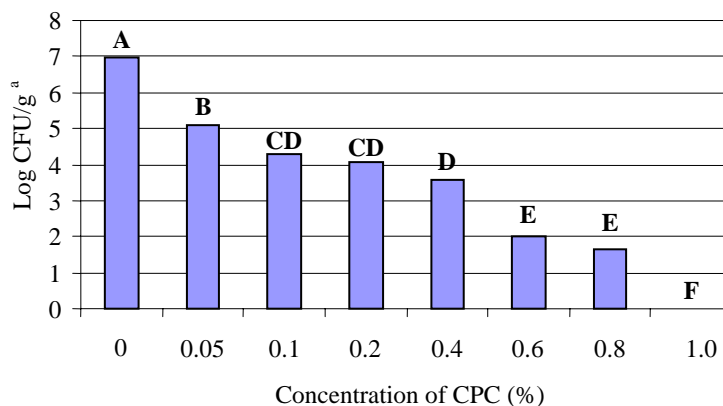


Figure 5: The effect of cetylpyridinium chloride against *L. monocytogenes* 1/2a on the surface of retail headless, shell-on, cooked shrimp without a water rinse stored at 4°C for 24 h.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

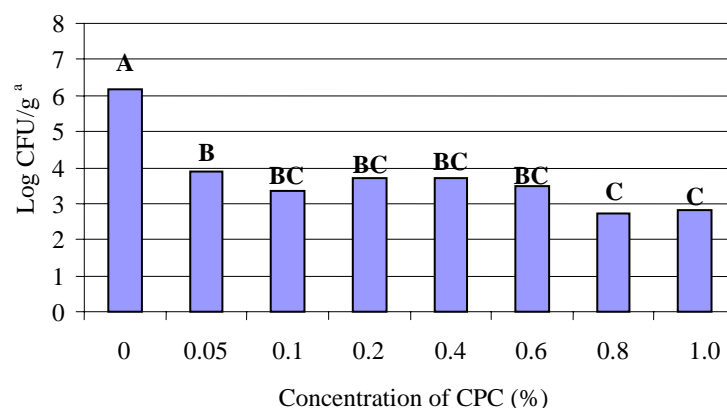


Figure 6: The effect of cetylpyridinium chloride against *L. monocytogenes* 1/2a on the surface of retail headless, shell-on, cooked shrimp with a water rinse stored at 4°C for 24h.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

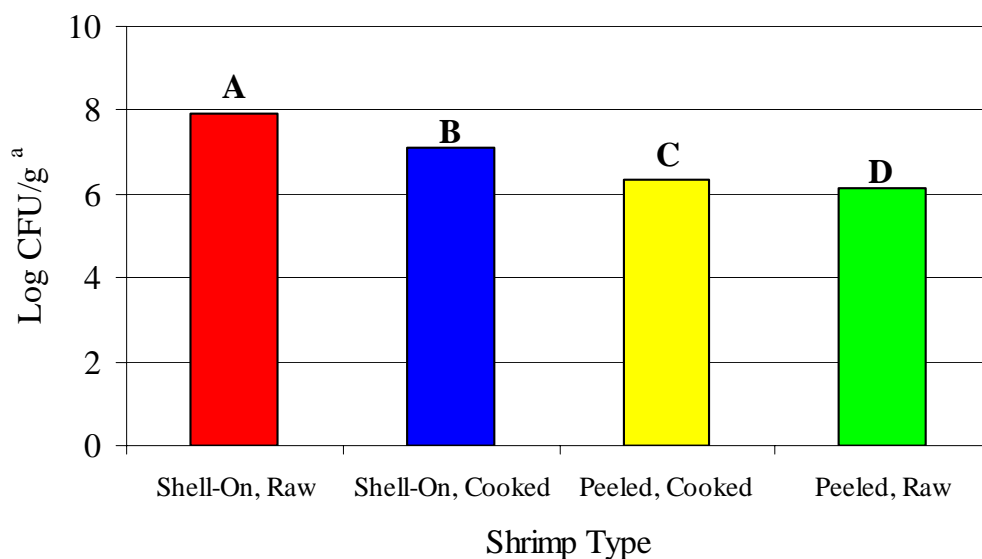


Figure 7: Attachment of *Listeria monocytogenes* 1/2a onto the surface of retail raw and cooked shrimp.

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

**Figure 1: The Effect of Cetylpyridinium Chloride against *L. monocytogenes* 1/2a on the Surface of Retail Headless, Shell-on, Raw Shrimp without a Water Rinse Stored at 4°C for 24h:** According to Figure 1, concentrations of CPC without a water rinse at 0.05, 0.1, 0.2, and 0.4% exhibited at least a 2 log CFU/g reduction in *L. monocytogenes* counts on headless, shell-on, raw shrimp purchased at retail. However, higher concentrations of 0.6 and 0.8% exhibited at least a 3 log CFU/g reduction in *L. monocytogenes* counts. In addition, it was observed that the most effective concentration of CPC was 1.0%, exhibiting approximately a 4.0 log CFU/g reduction in *L. monocytogenes* counts.

**Figure 2: The Effect of Cetylpyridinium Chloride against *L. monocytogenes* 1/2a on the Surface of Retail Headless, Shell-on, Raw Shrimp with a Water Rinse Stored at 4°C for 24h:** The most effective concentration of CPC with a water rinse that was treated on the surface headless, shell-on, raw shrimp was 1.0%, exhibiting a 3.0-log reduction. Other concentrations of 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8% CPC followed by a water rinse reduced *L. monocytogenes* counts on the surface of raw shrimp by about 2.5 logs (Figure 2).

**Figure 3: The Effect of Cetylpyridinium Chloride against *L. monocytogenes* 1/2a on the Surface of Retail Peeled, Raw Shrimp without a Water Rinse Stored at 4°C for 24 h:** The most effective concentration of CPC without a water rinse against *L. monocytogenes* on peeled, raw shrimp was 1.0%, exhibiting approximately a 3.0-log reduction (Figure 3). *Listeria monocytogenes* counts on the surface of peeled raw shrimp were reduced 1.0 log CFU/g with 0.05% CPC, 1.5 log CFU/g with 0.1 and 0.2% CPC, and 2.0 log CFU/g with 0.4, 0.6, and 0.8% CPC.

**Figure 4: The Effect of Cetylpyridinium Chloride against *L. monocytogenes* 1/2a on the Surface of Retail Peeled, Raw Shrimp with a Water Rinse Stored at 4°C for 24 h:** The most effective concentration of CPC against *L. monocytogenes* was 1.0%, with a 1.8-log reduction. *Listeria monocytogenes* counts on the surface of peeled raw shrimp with a water rinse were reduced by 1.0 to 1.5 log CFU/g with 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8% CPC.

**Figure 5: The Effect of Cetylpyridinium Chloride against *L. monocytogenes* 1/2a on the Surface of Retail Headless, Shell-on, Cooked Shrimp without a Water Rinse Stored at 4°C for 24 h:** According to Figure 5, it was observed that the most effective concentration of CPC without a water rinse on retail cooked shrimp was 1.0%, exhibiting approximately a 7 log CFU/g reduction. Of the all the retail shrimp tested, this was the most significant finding



because *L. monocytogenes* counts at 1.0% CPC were reduced to non-detectable levels. *L. monocytogenes* counts on the surface of cooked shrimp were reduced 1.0 log CFU/g with 0.05% CPC, 3.0 log CFU/g with 0.1, 0.2 and 0.4% CPC, 5.0 log CFU/g with 0.6%, and 6.0 log CFU/g with 0.8% CPC.

**Figure 6: The Effect of Cetylpyridinium Chloride against *L. monocytogenes* 1/2a on the Surface of Retail Headless, Shell-on, Cooked Shrimp with a Water Rinse Stored at 4°C for 24 h:** When a water rinse was performed, *L. monocytogenes* counts on the surface of cooked shrimp were reduced from 2.0 to 2.5 log CFU/g with 0.05, 0.1, 0.2, 0.4, and 0.6% CPC, and 3.0 log CFU/g with 0.8% and 1.0% CPC (Figure 6).

**Figure 7: Attachment of *Listeria monocytogenes* 1/2a onto the Surface of Retail Raw and Cooked Shrimp:** It was observed that the initial *L. monocytogenes* counts on the control samples for each shrimp type were significantly different from each other (Figure 7). The initial *L. monocytogenes* counts for the shell-on raw shrimp were 7.94 log CFU/g, the shell-on cooked shrimp were 7.09 log CFU/g, the peeled cooked shrimp were 6.37 log CFU/g, and the peeled raw shrimp were 6.15 log CFU/g.

Table 1: Antimicrobial effect of cetylpyridinium chloride without a water rinse against *Listeria monocytogenes* 1/2a on the surface of domestic white shrimp stored at 4°C for 9 days.

|                               |                           | Log CFU/g <sup>a</sup> |                |                |                 |
|-------------------------------|---------------------------|------------------------|----------------|----------------|-----------------|
|                               | Conc. of CPC <sup>b</sup> | Day 0                  | Day 3          | Day 6          | Day 9           |
| Shell-on, Raw <sup>c</sup>    | 0%                        | 7.13 ± 0.04 A          | 7.29 ± 0.13 A  | 7.61 ± 0.03 A  | 7.77 ± 0.04 B   |
|                               | 0.05%                     | 5.71 ± 0.04 C          | 6.03 ± 0.03 D  | 6.27 ± 0.04 D  | 6.60 ± 0.07 C   |
|                               | 0.4%                      | 5.18 ± 0.03 DE         | 5.40 ± 0.07 E  | 6.14 ± 0.04 DE | 6.36 ± 0.04 DE  |
|                               | 1.0%                      | 4.81 ± 0.21 F          | 5.23 ± 0.07 EF | 6.06 ± 0.06 EF | 6.14 ± 0.04 EFG |
| Shell-on, Cooked <sup>c</sup> | 0%                        | 7.13 ± 0.03 A          | 7.01 ± 0.04 B  | 7.35 ± 0.08 B  | 8.13 ± 0.04 A   |
|                               | 0.05%                     | 5.41 ± 0.07 DE         | 5.21 ± 0.13 EF | 5.43 ± 0.06 G  | 6.35 ± 0.08 DF  |
|                               | 0.4%                      | 5.07 ± 0.07 EF         | 5.03 ± 0.04 FG | 4.88 ± 0.04 H  | 6.15 ± 0.04 EFG |
|                               | 1.0%                      | 4.13 ± 0.33 G          | 4.60 ± 0.13 H  | 4.80 ± 0.06 H  | 6.04 ± 0.06 GH  |
| Peeled, Raw <sup>c</sup>      | 0%                        | 6.27 ± 0.12 B          | 6.73 ± 0.12 C  | 7.09 ± 0.13 C  | 7.90 ± 0.23 AB  |
|                               | 0.05%                     | 5.16 ± 0.04 DE         | 5.25 ± 0.17 E  | 6.11 ± 0.06 E  | 6.68 ± 0.07 C   |
|                               | 0.4%                      | 4.95 ± 0.07 EF         | 4.82 ± 0.03 G  | 5.96 ± 0.06 F  | 6.46 ± 0.04 CD  |
|                               | 1.0%                      | 4.18 ± 0.02 G          | 4.40 ± 0.02 H  | 5.47 ± 0.06 G  | 5.93 ± 0.22 GH  |

<sup>a</sup> All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means within each vertical column followed by the same letter are not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

<sup>b</sup> Different concentrations of cetylpyridinium chloride solutions (CPC) used to treat *L. monocytogenes*-inoculated shrimp samples. The shrimp samples were then washed with CPC on day 0 and then stored at 4°C.

<sup>c</sup> Three types of domestic shrimp were used during this study: (1) headless, shell-on raw shrimp, (2) headless, shell-on cooked; and (3) peeled, raw shrimp meat.

As observed in the 9-day shelf-life study at 4°C (Table 1), CPC washing significantly reduced *L. monocytogenes* counts on all inoculated shrimp types when compared to the control samples, regardless of the concentrations of CPC solutions. On day 9, the average *L. monocytogenes* counts for the shell-on, raw shrimp at 0% CPC was 7.77 log CFU/g, which was significantly higher than CPC-treated samples that had 6.60 (0.05%), 6.36 (0.4%), and 6.14 (1.0%) logs, respectively. Also, at day 9, the average *L. monocytogenes* counts for the shell-on, cooked shrimp at 0% CPC was 8.13 log CFU/g, which was significantly higher than CPC-treated samples that had 6.35 (0.05%), 6.15 (0.4%), and 6.04 (1.0%) logs, respectively. Likewise, at day 9, the average *L. monocytogenes* counts for the peeled, raw shrimp at 0% CPC was 7.90 log CFU/g, which was significantly higher than CPC-treated samples that had 6.68 (0.05%), 6.46 (0.4%), and 5.93 (1.0%) logs, respectively.

As observed in the 9-day shelf-life study at 4°C (Table 2), CPC washing followed by a water rinse significantly reduced *L. monocytogenes* counts on all inoculated shrimp types when compared to the control samples, regardless of the concentrations of CPC solutions. On day 9, the average *L. monocytogenes* counts for the shell-on, raw shrimp at 0% CPC was 7.65 log CFU/g, which was significantly higher than CPC-treated samples that had 6.77 (0.05%), 6.35 (0.4%), and 6.14 (1.0%) logs, respectively. Also, at day 9, the average *L. monocytogenes* counts for the shell-on, cooked shrimp at 0% CPC was 7.66 log CFU/g, which was significantly higher than CPC-treated samples that had 6.39 (0.05%), 6.20 (0.4%), and 6.04 (1.0%) logs, respectively. Likewise, at day 9, the average *L. monocytogenes* counts for the peeled, raw shrimp at 0% CPC was 7.48 log CFU/g, which was significantly higher than CPC-treated samples that had 6.37 (0.05%), 6.30 (0.4%), and 6.13 (1.0%) logs, respectively.

Table 2: Antimicrobial effect of cetylpyridinium chloride with a water rinse against *Listeria monocytogenes* 1/2a on the surface of domestic white shrimp stored at 4°C for 9 days.

| Log CFU/g <sup>a</sup>              |                           |               |                 |                |                 |
|-------------------------------------|---------------------------|---------------|-----------------|----------------|-----------------|
|                                     | Conc. of CPC <sup>b</sup> | Day 0         | Day 3           | Day 6          | Day 9           |
| <b>Shell-on, Raw<sup>c</sup></b>    | <b>0%</b>                 | 6.37 ± 0.03 A | 6.69 ± 0.12 A   | 6.98 ± 0.11 B  | 7.65 ± 0.08 A   |
|                                     | <b>0.05%</b>              | 5.71 ± 0.02 B | 5.86 ± 0.06 B   | 6.15 ± 0.04CDE | 6.77 ± 0.02 C   |
|                                     | <b>0.4%</b>               | 5.39 ± 0.04 C | 5.76 ± 0.04 B   | 6.03 ± 0.11 E  | 6.35 ± 0.04 DE  |
|                                     | <b>1.0%</b>               | 5.32 ± 0.04 C | 5.11 ± 0.06 DEF | 5.63 ± 0.06 F  | 6.14 ± 0.14 FH  |
| <b>Shell-on, Cooked<sup>c</sup></b> | <b>0%</b>                 | 6.38 ± 0.03 A | 6.80 ± 0.25 A   | 7.46 ± 0.03 CD | 7.66 ± 0.08 A   |
|                                     | <b>0.05%</b>              | 5.02 ± 0.07 D | 5.24 ± 0.09 CD  | 6.36 ± 0.08 F  | 6.39 ± 0.10 D   |
|                                     | <b>0.4%</b>               | 4.25 ± 0.03 F | 4.86 ± 0.20 FG  | 5.71 ± 0.09 G  | 6.20 ± 0.08 EFG |
|                                     | <b>1.0%</b>               | 4.19 ± 0.02 F | 4.81 ± 0.16 G   | 5.05 ± 0.08 I  | 6.04 ± 0.06 GH  |
| <b>Peeled, Raw<sup>c</sup></b>      | <b>0%</b>                 | 5.66 ± 0.08B  | 5.95 ± 0.08 B   | 6.47 ± 0.08 A  | 7.48 ± 0.05 B   |
|                                     | <b>0.05%</b>              | 5.32 ± 0.02 C | 5.41 ± 0.11 C   | 5.67 ± 0.30 DE | 6.37 ± 0.05 D   |
|                                     | <b>0.4%</b>               | 5.11 ± 0.01 D | 5.19 ± 0.13 CE  | 5.31 ± 0.04 F  | 6.30 ± 0.02 DF  |
|                                     | <b>1.0%</b>               | 4.86 ± 0.13 E | 4.91 ± 0.04 EG  | 5.05 ± 0.09 I  | 6.13 ± 0.04 GH  |

<sup>a</sup> All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means within each vertical column followed by the same letter are not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

<sup>b</sup> Different concentrations of cetylpyridinium chloride solutions (CPC) used to treat *L. monocytogenes*-inoculated shrimp samples. The shrimp samples were then washed with CPC on day 0, treated with water rinse, and then stored at 4°C.

<sup>c</sup> Three types of domestic shrimp were used during this study: (1) headless, shell-on raw shrimp, (2) headless, shell-on cooked; and (3) peeled, raw shrimp meat.

Table 3: Antimicrobial effect of cetylpyridinium chloride against *Listeria monocytogenes* 1/2a biofilm on the surface of domestic white shrimp stored at 4°C for 8 days.

|                                     |                           | Log CFU/g <sup>a</sup> |               |               |               |               |
|-------------------------------------|---------------------------|------------------------|---------------|---------------|---------------|---------------|
|                                     | Conc. of CPC <sup>b</sup> | Day 0                  | Day 2         | Day 4         | Day 6         | Day 8         |
| <b>Shell-on, Raw<sup>c</sup></b>    | <b>0%</b>                 | 6.67 ± 0.20A           | 7.13 ± 0.03A  | 7.38 ± 0.02A  | 7.45 ± 0.13A  | 7.46 ± 0.04 B |
|                                     | <b>0.05%</b>              | 5.19 ± 0.20 C          | 6.41 ± 0.03B  | 6.46 ± 0.18D  | 6.65 ± 0.08 B | 7.16 ± 0.04 C |
|                                     | <b>0.4%</b>               | 4.66 ± 0.20D           | 6.09 ± 0.12C  | 6.39 ± 0.09D  | 6.49±0.02BC   | 6.80 ± 0.14D  |
|                                     | <b>1.0%</b>               | 4.09 ± 0.20 E          | 5.65±0.33DE   | 5.44 ± 0.04 F | 5.50 ± 0.28 F | 6.08 ± 0.13 E |
| <b>Shell-on, Cooked<sup>c</sup></b> | <b>0%</b>                 | 6.14 ± 0.08 B          | 7.25 ± 0.02A  | 7.47 ± 0.19A  | 7.41 ± 0.09A  | 7.87 ± 0.04A  |
|                                     | <b>0.05%</b>              | 4.51±0.37DE            | 5.83±0.10CD   | 6.25 ± 0.03D  | 6.21±0.16CD   | 6.22 ± 0.12 E |
|                                     | <b>0.4%</b>               | 4.08 ± 0.18 E          | 4.73 ± 0.18 F | 5.20 ± 0.09G  | 5.74 ± 0.08 F | 5.42 ± 0.16 F |
|                                     | <b>1.0%</b>               | 2.44 ± 0.05 F          | 3.15 ± 0.21G  | 4.37 ± 0.13H  | 4.46 ± 0.18G  | 4.50 ± 0.13G  |
| <b>Peeled, Raw<sup>c</sup></b>      | <b>0%</b>                 | 5.98 ± 0.11 B          | 6.98 ± 0.04A  | 7.12 ± 0.03 B | 7.37 ± 0.15A  | 7.45 ± 0.21 B |
|                                     | <b>0.05%</b>              | 5.17 ± 0.02 C          | 6.03 ± 0.11 C | 6.72 ± 0.04 C | 6.70 ± 0.28 B | 6.90 ± 0.01D  |
|                                     | <b>0.4%</b>               | 4.78±0.10CD            | 5.35 ± 0.13 E | 6.41 ± 0.03D  | 6.12±0.08DE   | 6.29 ± 0.04 E |
|                                     | <b>1.0%</b>               | 4.19 ± 0.05 E          | 5.01 ± 0.08 F | 5.81 ± 0.04 E | 5.83 ±0.19EF  | 5.42 ± 0.13 F |

<sup>a</sup> All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means within each vertical column followed by the same letter are not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

<sup>b</sup> Different concentrations of cetylpyridinium chloride solutions (CPC) used to treat *L. monocytogenes*-inoculated shrimp samples and then stored at 4°C.

<sup>c</sup> Three types of domestic shrimp were used during this study: (1) headless, shell-on raw shrimp, (2) headless, shell-on cooked; and (3) peeled, raw shrimp meat.

As observed in the 8-day biofilm study at 4°C (Table 3), CPC washing significantly reduced *L. monocytogenes* counts on all inoculated shrimp types when compared to the control samples, regardless of the concentrations of CPC solutions. On day 8, the average *L.*

*monocytogenes* counts for the shell-on, raw shrimp at 0% CPC was 7.46 log CFU/g, which was significantly higher than CPC-treated samples that had 7.16 (0.05%), 6.80 (0.4%), and 6.08 (1.0%) logs, respectively. Also, at day 8, the average *L. monocytogenes* counts for the shell-on, cooked shrimp at 0% CPC was 7.87 log CFU/g, which was significantly higher than CPC-treated samples that had 6.22 (0.05%), 5.42 (0.4%), and 4.50 (1.0%) logs, respectively. Likewise, at day 8, the average *L. monocytogenes* counts for the peeled, raw shrimp at 0% CPC was 7.45 log CFU/g, which was significantly higher than CPC-treated samples that had 6.90 (0.05%), 5.29 (0.4%), and 5.42 (1.0%) logs, respectively.

By day 8, *L. monocytogenes* counts that were exposed to 1.0% CPC were significantly reduced by 1.38 logs for shell-on raw shrimp, 2.03 logs for peeled raw shrimp, and 3.11 logs for shell-on cooked shrimp.

During our study, when *L. monocytogenes*-inoculated shrimp samples were exposed to 0.05%, 0.4%, and 1.0% CPC then stored at -20°C for 90 day, all concentrations of CPC were significantly lower than the controls, regardless of shrimp type (Table 4). Reductions of *L. monocytogenes* counts on CPC-treated shrimp were 0.66 to 1.07 logs, 0.94 to 1.69 logs, and 0.46 to 0.77 logs for shell-on raw shrimp, shell-on cooked shrimp, and peeled raw shrimp, respectively.

When *L. monocytogenes*-inoculated CPC-treated shrimp were exposed to a water rinse then stored at -20°C for 90 days, all concentrations of CPC were significantly lower than the controls, regardless of shrimp type. *L. monocytogenes* counts were reduced by 0.77 to 1.29 logs on shell-on raw shrimp, 1.15 to 2.25 logs on the shell-on cooked shrimp, 0.48 to 1.06 logs on peeled raw shrimp when the shrimp samples were treated with 0.05, 0.4, or 1.0% CPC followed by a water rinse (Table 5).

Table 4: Antimicrobial effect of cetylpyridinium chloride without a water rinse against *Listeria monocytogenes* 1/2a on the surface of domestic white shrimp stored at -20°C for 90 days.

|                                     |                           | Log CFU/g <sup>a</sup> |               |                |               |               |                |                |
|-------------------------------------|---------------------------|------------------------|---------------|----------------|---------------|---------------|----------------|----------------|
|                                     | Conc. of CPC <sup>b</sup> | Day 0                  | Day 7         | Day 14         | Day 21        | Day 30        | Day 60         | Day 90         |
| <b>Shell-on, Raw<sup>c</sup></b>    | <b>0%</b>                 | 7.03 ± 0.11 A          | 6.88 ± 0.04 A | 7.20 ± 0.09 A  | 7.33 ± 0.02 A | 7.05 ± 0.04 A | 7.02 ± 0.03 A  | 6.39 ± 0.04 B  |
|                                     | <b>0.05%</b>              | 5.64 ± 0.07 C          | 6.31 ± 0.01 B | 6.11 ± 0.04 B  | 6.22 ± 0.17 B | 5.58 ± 0.13 D | 5.68 ± 0.04 D  | 5.73 ± 0.04 D  |
|                                     | <b>0.4%</b>               | 5.18 ± 0.03 E          | 5.60 ± 0.14 E | 5.78 ± 0.04 C  | 5.33±0.03 DE  | 5.36 ± 0.06 E | 5.48 ±0.23DF   | 5.55 ±0.09 EF  |
|                                     | <b>1.0%</b>               | 4.14 ± 0.18 E          | 5.27 ± 0.01 F | 5.45 ± 0.03DEF | 5.11 ±0.10 EF | 5.28 ±0.03EG  | 5.20 ±0.08FG   | 5.32 ±0.03FGH  |
| <b>Shell-on, Cooked<sup>c</sup></b> | <b>0%</b>                 | 7.13 ± 0.04 A          | 6.97 ± 0.04 A | 7.31 ± 0.08 A  | 7.40 ± 0.08 A | 6.56 ± 0.08 B | 6.67 ± 0.05 B  | 6.67 ± 0.08 A  |
|                                     | <b>0.05%</b>              | 5.21 ± 0.01 D          | 6.03±0.03 CD  | 5.57±0.19 DE   | 5.78 ± 0.04 C | 5.35 ± 0.04EF | 5.53 ±0.19DF   | 5.73 ± 0.06 D  |
|                                     | <b>0.4%</b>               | 5.07 ± 0.07 D          | 5.89 ± 0.06 D | 5.31±0.08 EF   | 5.55±0.15 CD  | 5.25 ±0.04EH  | 5.19 ±0.04FH   | 5.15 ±0.04 HI  |
|                                     | <b>1.0%</b>               | 4.13 ± 0.33 E          | 4.97 ± 0.06 G | 4.45 ± 0.06 G  | 4.96 ± 0.04 F | 5.20±0.02FGHI | 5.04±0.06GH    | 4.98 ± 0.03 I  |
| <b>Peeled, Raw<sup>c</sup></b>      | <b>0%</b>                 | 6.36 ± 0.06 B          | 7.13 ± 0.07 A | 7.42 ± 0.03 A  | 7.32 ± 0.07 A | 7.14 ± 0.08 A | 6.32 ± 0.03 C  | 5.92 ± 0.18 C  |
|                                     | <b>0.05%</b>              | 5.21 ± 0.09 D          | 6.11 ± 0.01 C | 6.12 ± 0.13 B  | 5.77 ± 0.11 C | 5.80 ± 0.08 C | 5.58 ±0.09DE   | 5.46 ± 0.08EFG |
|                                     | <b>0.4%</b>               | 4.34 ± 0.01 E          | 5.55 ± 0.20 E | 5.33±0.03 EF   | 5.47 ± 0.14 D | 5.54 ± 0.13 D | 5.40 ± 0.12EFH | 5.26±0.08GH    |
|                                     | <b>1.0%</b>               | 4.18 ± 0.02 E          | 5.47 ± 0.11 E | 5.18 ± 0.04 F  | 5.17 ±0.17 EF | 5.21 ± 0.04EI | 5.07±0.23GH    | 5.15 ± 0.04 H  |

(table con'd.)

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means within each vertical column followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

<sup>b</sup> Different concentrations of cetylpyridinium chloride solutions (CPC) used to treat *L. monocytogenes*-inoculated shrimp samples. The shrimp samples were then washed with CPC on day 0 and then stored at 4°C.

<sup>c</sup> Three types of domestic shrimp were used during this study: (1) headless, shell-on raw shrimp, (2) headless, shell-on cooked; and (3) peeled, raw shrimp meat.



Table 5: Antimicrobial effect of cetylpyridinium chloride with a water rinse against *Listeria monocytogenes* 1/2a on the surface of domestic white shrimp stored at -20°C for 90 days.

|   |                              | Log CFU/g <sup>a</sup> |               |                |               |               |               |                |
|---|------------------------------|------------------------|---------------|----------------|---------------|---------------|---------------|----------------|
|   | Conc.<br>Of CPC <sup>b</sup> | Day 0                  | Day 7         | Day 14         | Day 21        | Day 30        | Day 60        | Day 90         |
| <b>Shell-on,<br/>Raw<sup>c</sup></b>    | <b>0%</b>                    | 7.12 ± 0.06 A          | 7.15 ± 0.07 A | 7.17 ± 0.05 B  | 6.67 ± 0.04 C | 6.65 ± 0.12 A | 6.56 ± 0.04 A | 6.51 ± 0.15 A  |
|   | <b>0.05%</b>                 | 6.18 ± 0.05 B          | 5.96 ± 0.07 C | 5.79 ± 0.07 C  | 5.75 ± 0.08DE | 5.82 ± 0.15CD | 5.87 ± 0.15 C | 5.76 ± 0.08 C  |
|   | <b>0.4%</b>                  | 5.35 ± 0.15CE          | 5.42 ± 0.03 E | 5.37 ± 0.06DE  | 5.37 ± 0.10FG | 5.51 ± 0.18 E | 5.40 ± 0.01DE | 5.46 ± 0.10DE  |
|   | <b>1.0%</b>                  | 5.34 ± 0.04CF          | 5.27 ± 0.04EF | 5.27 ± 0.01DG  | 5.14 ± 0.01 H | 5.19 ± 0.02 F | 5.09 ± 0.07 F | 5.22 ± 0.02FG  |
| <b>Shell-on,<br/>Cooked<sup>c</sup></b> | <b>0%</b>                    | 7.25 ± 0.03 A          | 7.09 ± 0.05 A | 7.42 ± 0.20 A  | 7.14 ± 0.08 B | 6.37 ± 0.04 B | 6.24 ± 0.14 B | 6.48 ± 0.06 A  |
|   | <b>0.05%</b>                 | 5.40 ± 0.07 C          | 6.05 ± 0.05BC | 5.95 ± 0.02DE  | 5.91 ± 0.15 D | 5.59 ± 0.13DE | 5.47 ± 0.09 D | 5.33 ± 0.11EF  |
|   | <b>0.4%</b>                  | 5.23 ± 0.01DEF         | 5.71 ± 0.15 D | 5.23 ± 0.01EFG | 5.27 ± 0.03FH | 5.38 ± 0.11EF | 5.20 ± 0.04EF | 4.92 ± 0.04 I  |
|   | <b>1.0%</b>                  | 4.29 ± 0.09 H          | 5.06 ± 0.04 G | 5.07 ± 0.07EFG | 5.18 ± 0.04GH | 4.63 ± 0.17 G | 4.42 ± 0.08 G | 4.23 ± 0.08 J  |
| <b>Peeled,<br/>Raw<sup>c</sup></b>      | <b>0%</b>                    | 6.30 ± 0.04 B          | 7.06 ± 0.01 A | 7.23 ± 0.02 B  | 7.54 ± 0.18 A | 6.22 ± 0.11 B | 6.22 ± 0.09 B | 6.12 ± 0.11 B  |
|   | <b>0.05%</b>                 | 5.36 ± 0.05CD          | 6.15 ± 0.06 B | 5.42 ± 0.07 D  | 5.68 ± 0.17 E | 5.87 ± 0.08 C | 5.76 ± 0.04 C | 5.64 ± 0.16CD  |
|   | <b>0.4%</b>                  | 5.08 ± 0.02 G          | 5.61 ± 0.12 D | 5.30 ± 0.03DF  | 5.45 ± 0.10 F | 5.37 ± 0.06EF | 5.23 ± 0.04EF | 5.14 ± 0.01FH  |
|   | <b>1.0%</b>                  | 4.94 ± 0.07 G          | 5.17 ± 0.03FG | 5.23 ± 0.04EFG | 5.22 ± 0.04GH | 5.25 ± 0.06 F | 5.13 ± 0.03 F | 5.06 ± 0.02GHI |

(table con'd.)

<sup>a</sup> All analyses were based on two separate experiments with each mean  $\pm$  standard deviation being average of two determinations. Means within each vertical column followed by the same letter are not significantly different ( $P = 0.05$ ) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

<sup>b</sup> Different concentrations of cetylpyridinium chloride solutions (CPC) used to treat *L. monocytogenes*-inoculated shrimp samples. The shrimp samples were then washed with CPC on day 0, treated with water rinse, and then stored at 4°C.

<sup>c</sup> Three types of domestic shrimp were used during this study: (1) headless, shell-on raw shrimp, (2) headless, shell-on cooked; and (3) peeled, raw shrimp meat.

## DISCUSSION

As observed in our studies of the effect of CPC against *Listeria monocytogenes*-inoculated retail shrimp (Figures 1-6), the most effective concentration of CPC was 1.0%. This coincides with the idea that CPC is concentration-dependent (Lim and Mustapha, 2004; and Wang and others, 2001). For the *L. monocytogenes* –inoculated shrimp that were treated with 1.0% CPC without a water rinse, *L. monocytogenes* counts were reduced between 3.0 to 7.0 logs after 24h (Figures 1, 3, 5). Overall, the highest reduction of *L. monocytogenes* was found on the surface of retail headless, shell-on cooked shrimp, resulting in non-detectable levels. However, variations were observed when these shrimp samples were treated with a water rinse (Figures 2, 4, 6). When comparing the water rinse treatment to the *L. monocytogenes* counts under the treatment without a water rinse, it was observed that the reductions were slightly higher. To our knowledge, there is no published evidence to support these discoveries. An explanation for this could have resulted from the water rinse washing some CPC away from the shrimp surface, thereby reducing the antimicrobial effect of CPC against *L. monocytogenes* (Figures 2, 4, 6).

As observed in the 9-day shelf-life study at 4°C (Table 1), CPC washing significantly reduced *L. monocytogenes* counts on all inoculated shrimp types when compared to the control samples, regardless of the concentrations of CPC solutions. The highest reduction of *L. monocytogenes* counts were found on the surface of domestic headless, shell-on cooked shrimp, exhibiting a 2.09-log reduction. However, this reduction was lower than the reduction found on the surface of retail, headless, shell-on cooked shrimp as seen in Figure 5. An explanation for this could have resulted from a possible synergistic effect between CPC and sodium tripolyphosphate found in retail shrimp, whereas, the domestic shrimp did not contain sodium tripolyphosphate.

The initial *L. monocytogenes* counts for the control shrimp samples were about 6.3 to 7.1 log CFU/g and grew to about 7.8 to 8.1 log CFU/g when stored at 4°C (Table 1). The same pattern was also observed when *L. monocytogenes*-inoculated shrimp samples were treated with a water rinse (Table 2). In a previous study by Mu and colleagues (1997), a similar growth pattern was observed. In their study, changes in *L. monocytogenes* population in shell-on raw shrimp increased from an initial count of 5.58 logs (day 0) to 8.52 logs (day 9) when stored at 4°C.

Our study indicates that the cells of *L. monocytogenes* formed a stronger attachment to the shells of shrimp than to the flesh of peeled shrimp meat. In the study by Mu and others (1997), a similar trend was also observed. In their study, the effect of trisodium phosphate (TSP) at concentrations of 10% or 20% against *L. monocytogenes* attached to shrimp during refrigerated storage was investigated. Headed white and brown shrimp (*Penaeus* spp.) were purchased from the Knight seafood Company in Brunswick, Georgia. *Listeria monocytogenes* counts of inoculated shrimp were evaluated after 0, 3, 6, and 9 days of storage at 4°C.

According to their work, *L. monocytogenes* counts were higher on shell-on shrimp compared to peeled shrimp. Mu and colleagues proposed two explanations for their findings. The first explanation given was that *L. monocytogenes* formed a strong attachment to shrimp shells. The second explanation given was that *L. monocytogenes* becomes physically entrapped once they were inoculated, and the attached or entrapped cells were not removed by water or TSP treatments. However, Mu and colleagues concluded that the mechanism of attachment or entrapment of *L. monocytogenes* cells was unknown and should be investigated in the future.

According to a more recent study by Dykes and others (2003), the quantification of *L. monocytogenes* levels on the shell and flesh of artificially contaminated cooked black tiger

prawns (*Penaeus monodon*) was examined. Whole cooked black tiger prawns with shells were purchased on ice from a local retail store and inoculated within sixty minutes of purchase with *L. monocytogenes* Scott A. However, their experimental design differed from our study. Their design consisted of two experimental protocols. The first protocol used simulated gross contamination of prawns with *L. monocytogenes* followed by immediate peeling. The second protocol simulated slight contamination followed by refrigerated storage before peeling, respectively. In both methods, their results exhibited a 0.90-log difference between *L. monocytogenes* attachment on shell-on cooked prawn versus peeled cooked prawns, which correlates closely to our findings of 0.75-log difference between shell-on cooked shrimp versus peeled cooked (Figure 7).

In addition to these observations, Dykes and colleagues (2003) further concluded in a preliminary study that *L. monocytogenes* counts on shell-on raw prawns differed to a greater degree than peeled raw prawns purchased at retail. In addition, the difference between *L. monocytogenes* counts on cooked shrimp versus raw shrimp also differed to a greater degree. Our study exhibited similar results showing approximately a 1.80-log difference between shell-on raw shrimp versus peeled raw shrimp purchased at retail (Figure 7).

When comparing the results of Dykes and colleagues (2003) to the results of our study, the most remarkable similarity was the approximate log differences between the shell-on shrimp and the peeled shrimp. It has been suggested that chitin, the main constituent of shrimp and prawn shells, enhances attachment of *L. monocytogenes* to shrimp (McCarthy, 1992).

In an FDA research study on raw shrimp, *L. monocytogenes* was found on the shells, but not in the digestive tract, even when exposed to high levels of *L. monocytogenes* in aquaculture tanks (Hatha and others, 2003; Van Wagner, 1989). Although aquaculture ponds are relatively

uncontaminated, periodic visits by aquatic birds may pose a threat of contaminating the system due to their droppings (Appendix 2). However, when *L. monocytogenes*-contaminated shrimp was boiled for one minute or less, the *L. monocytogenes* on the shells was easily killed (Van Wagner, 1989). Therefore, there is more concern for cooked shrimp than raw shrimp because the pathogen is in ready-to-eat form. According to researchers (Anonymous, 1987; McCarthy and others, 1990; and Van Wagner, 1989), evidence points to cross-contamination as the source of *Listeria* present on cooked and processed seafood. As a result of cross-contamination and increasing numbers of seafood imports from developing countries, the FDA implemented the Hazard Analysis Critical Control Point System (HACCP) in 1985 to combat cross-contamination of raw and processed foods. The FDA and other researchers have highlighted several critical control points that must be monitored in the seafood industry to help prevent contamination (Appendices 4 and 5). In general, the most important step in controlling bacteria in the production of seafood is by preventing the introduction of *L. monocytogenes* into the processing environment (Rorvik and others, 2000).

In a study conducted by McCarthy (1992), the attachment of *L. monocytogenes* to chitin and resistance to biocides were explored. The results demonstrated that quaternary ammonium compounds (QACs) were more effective for disinfecting *L. monocytogenes* cells attached to chitin flakes than iodine and chlorine. The QAC they used was dimethyl benzyl ammonium chloride. Exposure of attached cells to chitin flakes at 50 to 400 ppm of the QAC for one minute reduced *L. monocytogenes* counts by 1.0 to 2.0 logs. Similar results were observed in our study when *L. monocytogenes* cells attached to shell-on shrimp were exposed to 500 ppm (0.05%) of CPC for one minute, exhibiting 1.40 to 2.10-log reductions (Figure 1, Tables 1, 3, and 4).

During the same study, McCarthy (1992) also studied the efficacy of a dimethyl benzyl ammonium chloride against a 2-day and 7-day biofilm of *L. monocytogenes* cells attached to chitin. The results concluded that after treatment with the QAC, the 7-day biofilms were more resistant to disinfection than the 2-day biofilm. As illustrated in Table 3, our results further validate this idea resulting in a more complex biofilm after day 8 than at day 2 in all three shrimp types.

Likewise, Richards (1999) studied the efficacy of various sanitizers including various QACs against *L. monocytogenes* biofilms. Although the age of the biofilm was not stated, however, a mature biofilm was implied. According to Richards (1999), the biofilms were treated with QACs for two to five minutes. The results concluded that QACs were the most effective sanitizers against *L. monocytogenes* biofilms when compared to other sanitizers, reducing counts by 1.65 to 6.06 logs. Similar results were observed in our study (Table 3). By day 8, *L. monocytogenes* counts that were exposed to 1.0% CPC were significantly reduced by 1.38 logs for shell-on raw shrimp, 2.03 logs for peeled raw shrimp, and 3.11 logs for shell-on cooked shrimp.

As noted previously, there is a greater concern for *L. monocytogenes*-contaminated cooked shrimp than *L. monocytogenes*-contaminated raw shrimp because the pathogen is in the ready-to-eat form (Van Wagner, 1988). In a study conducted by Weagant and colleagues (1988), frozen shrimp obtained by the FDA Seattle District Laboratory were analyzed for *L. monocytogenes* contamination as a follow-up to earlier findings (Ryser, 1991). Of the 8 frozen cooked shrimp samples examined, 2 tested positive for *L. monocytogenes* serotypes 1a and 4b. In addition to this, of the 7 frozen raw shrimp samples examined, 2 tested positive for *L.*

*monocytogenes*. Therefore, we investigated the survival of *L. monocytogenes* on the surface of shrimp treated with CPC stored at freezer temperatures.

During our study, when *L. monocytogenes*-inoculated shrimp samples were exposed to 0.05%, 0.4%, and 1.0% CPC then stored at -20°C for 90 day, all concentrations of CPC were significantly different from the controls, regardless of shrimp type (Table 4). The highest reduction of *L. monocytogenes* counts on CPC-treated shrimp was 1.69 Log CFU/g found on the surface of headless, shell-on cooked shrimp. A similar pattern was observed when *L. monocytogenes*-inoculated CPC-treated shrimp were exposed to a water rinse then stored at -20°C for 90 days (Table 5). Again, results showed that at day 90 all concentrations of CPC were significantly different from the controls, regardless of shrimp type. The highest reduction of *L. monocytogenes* counts was also found on the surface of headless, shell-on cooked shrimp. In both of these studies, it was also observed that the initial *L. monocytogenes* counts of the control shrimp samples, regardless of shrimp type, were reduced when stored at -20°C (Tables 4 and 5).

Although *L. monocytogenes* counts were reduced on the surface of the domestic shrimp in some instance, CPC treatment was not effective in reducing *L. monocytogenes* counts to non-detectable levels as observed in the retail shrimp samples. In a second study conducted by McCarthy and colleagues (1990), similar results were observed. Their results showed that *L. monocytogenes* populations were enumerated from shell-on raw shrimp samples after storage at -20°C for 90 days. Therefore, it is evident that this pathogen may be resistant to subfreezing temperatures.



## CONCLUSION

Although the use of CPC as an antimicrobial agent for seafood has not been approved by the FDA, we have shown in this study the strong potential of cetylpyridinium chloride as a washing solution to reduce *L. monocytogenes* on the surface of raw and cooked shrimp. However, further investigation is necessary to determine its impact on sensory and physical properties such as color and texture of shrimp as well as determining CPC residuals on the surface of raw and cooked shrimp. To date, the use of CPC has only been approved by the FDA at a level not to exceed 0.3 grams of CPC and should also contain propylene glycol at a concentration of 1.5 times that of the CPC per pound of raw poultry carcass.

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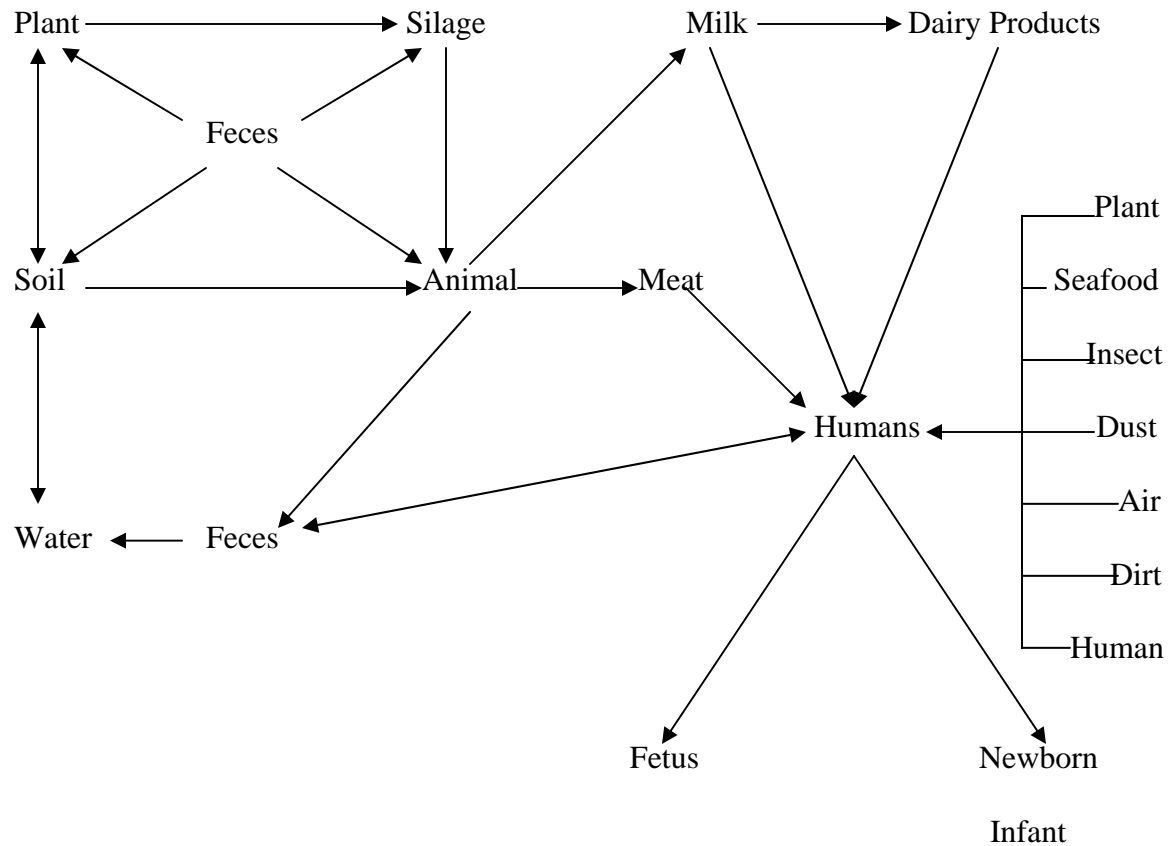
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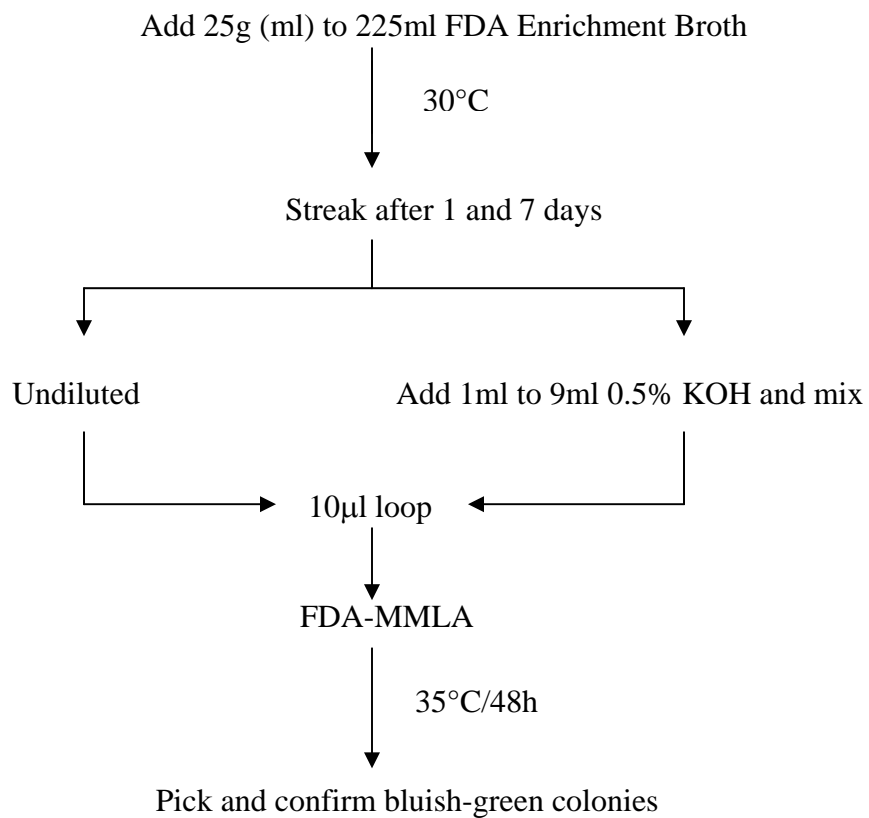
## APPENDIX 1

### ROUTES FOR TRANSMISSION OF *L. MONOCYTOGENES* TO HUMANS (RYSER, 1991)



## APPENDIX 2

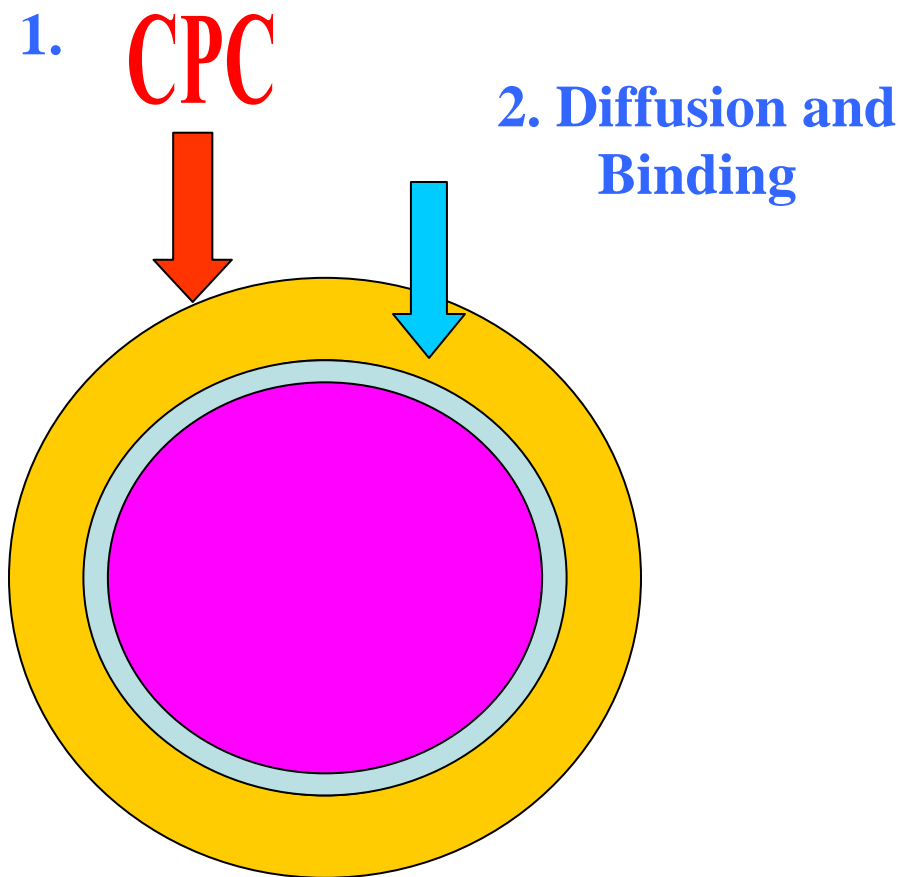
### ORIGINAL FDA PROCEDURE FOR ISOLATING *L. MONOCYTOGENES* FROM FOODS, JUNE 1985 TO OCTOBER 1988 (RYSER, 1991)



### APPENDIX 3

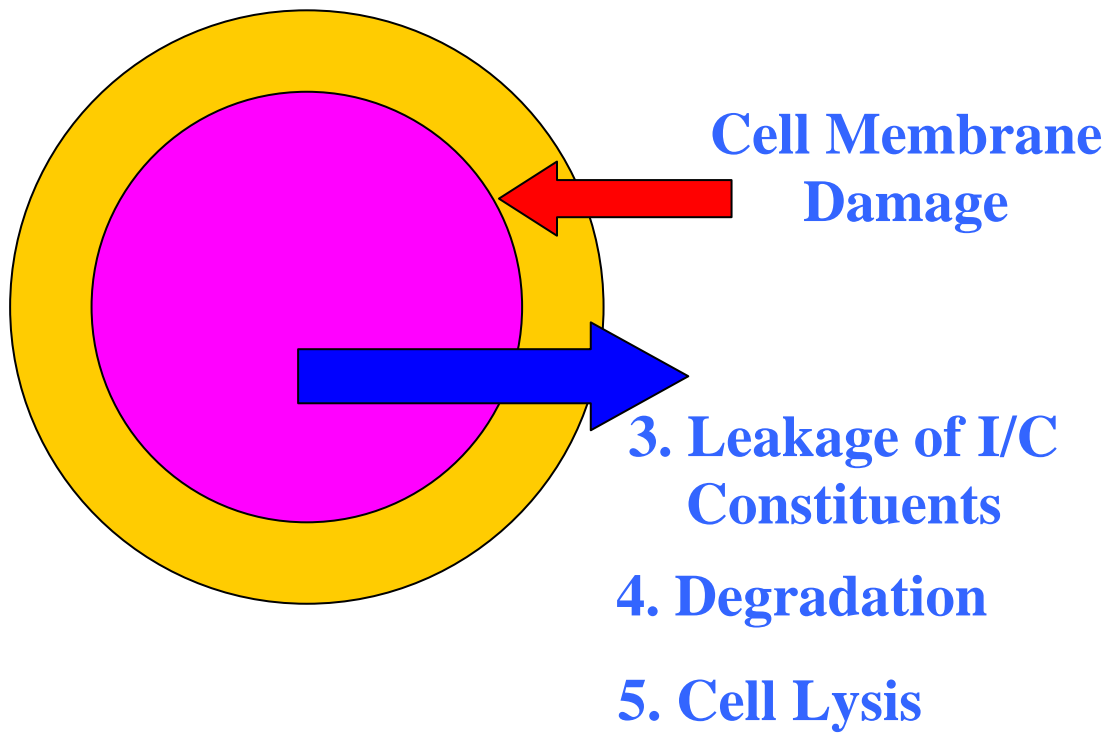
#### SEQUENCE OF EVENTS IN THE ACTION OF LYTIC AGENTS ON BACTERIA (SALTON, 1968)

##### General Uptake





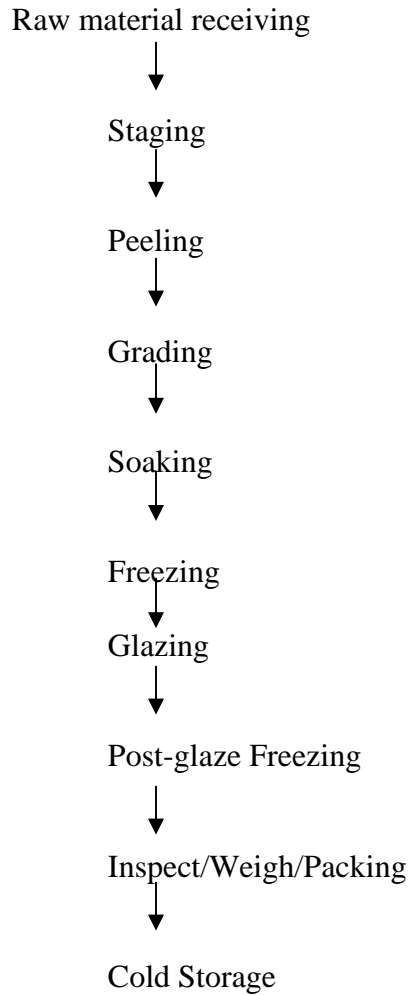
Cell Damage



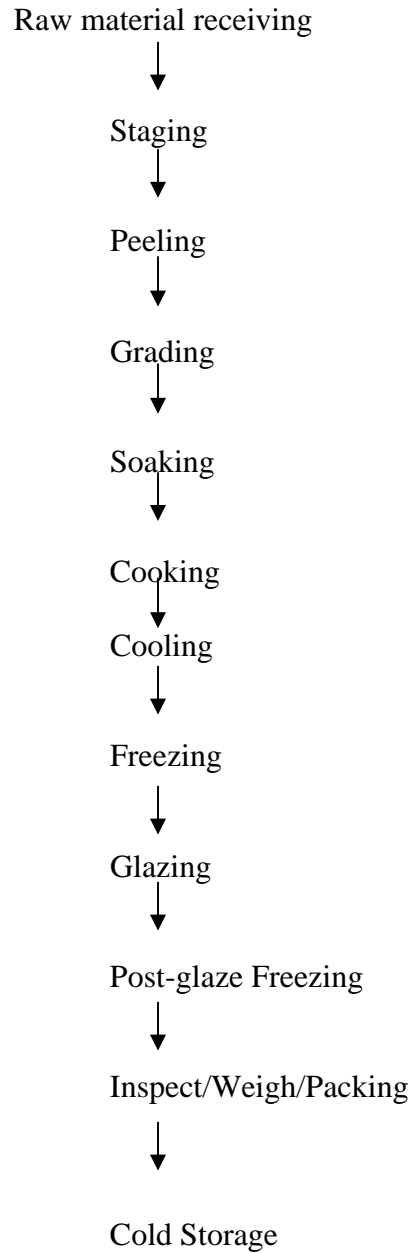
## APPENDIX 4

### PROCESS FLOW FOR THE RAW AND COOKED SHRIMP (MOHAMED HAHTA AND OTHERS, 2003)

#### Process Flow for Raw Shrimp



#### Process Flow for Cooked Shrimp



## APPENDIX 5

### CRITICAL CONTROL POINTS THAT MUST BE MONITORED IN THE SEAFOOD INDUSTRY TO HELP PREVENT CONTAMINATION (VAN WAGNER, 1989)

#### HACCP Plan Requirements:

- Identify and assess the components of processing
- Determine the critical control points
- Establish monitoring of critical control points
- Call for technical expertise
- Train your workers in HACCP procedures

#### Sources of Problems:

##### *Probable contamination factors*

- Improper handling of foods after cooking
- Infrequent breakdown and cleaning of equipment
- Inadequate plant design to separate raw product
- Indifferent employee attitude toward sanitation

##### *Problems in the Plant*

- Refrigerator areas – temperature and separation of product
- Cleanliness of walls, floors, and ceilings

##### *Problems in Processing Areas*

- Pooled water/splash back
- Processing wastes

## Solutions

### *Control Plant Traffic*

- Restrict access to areas
- Make sure workers are clean
- Segregate personnel who work with raw foods from those who work with processed foods
- Use specific equipment for raw and processed product
- Ensure workers wear designated plant clothing

### *Sampling & Testing*

- Coliforms as an index of contamination
- Environmental sampling
- Testing in isolation

### *Prevent airborne contamination by eliminating*

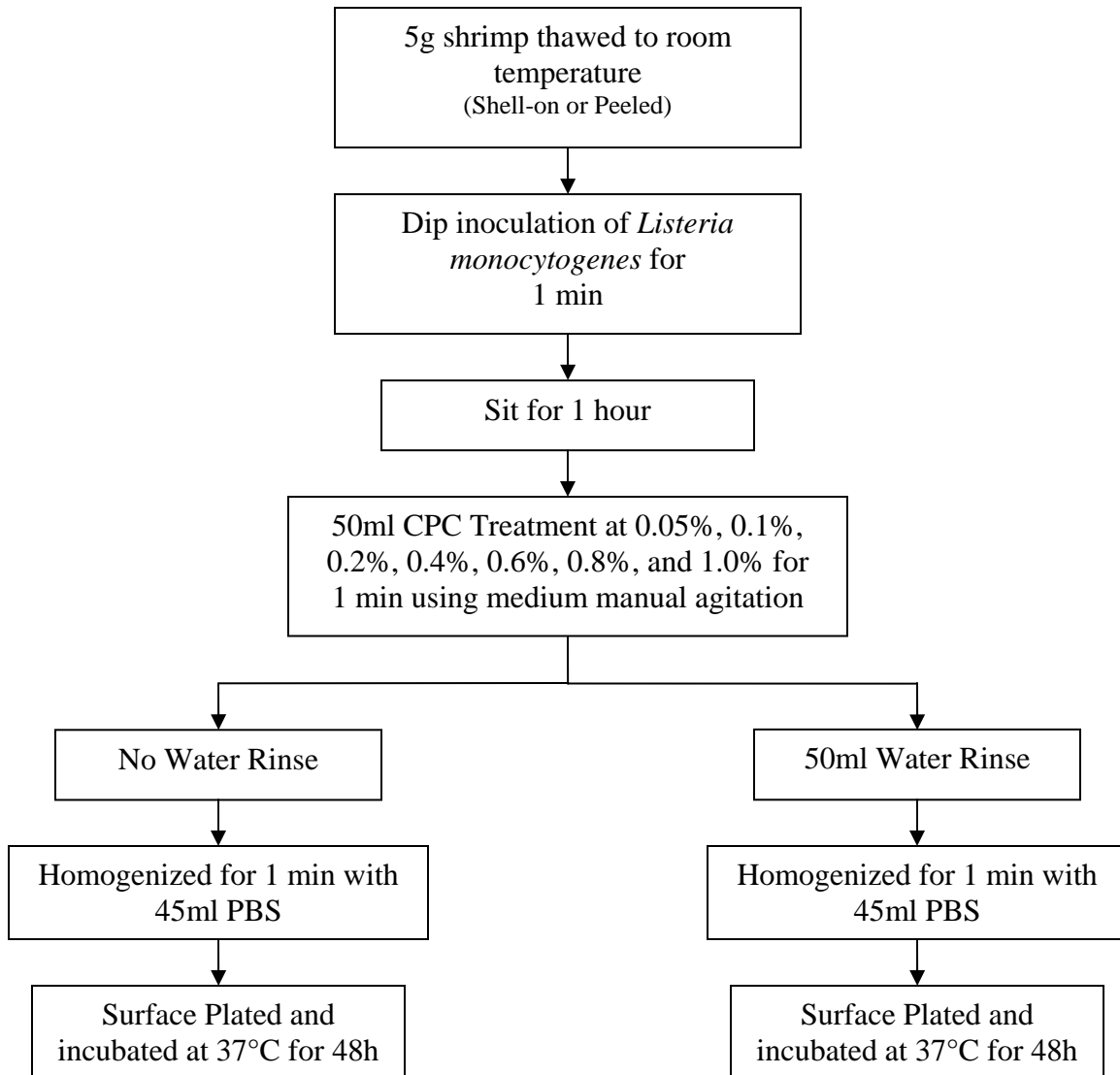
- Drip condensate
- High-pressure hose
- Floor drains near packaging areas
- Air transfer systems from raw to finished packaging areas

### *Cleaning & Sanitation*

- Routinely sanitize floor drains – don't use high pressure hose
- Pay attention to conveyor tracks and belts
- Avoid rags and sponges
- Color-code brushes for intended use

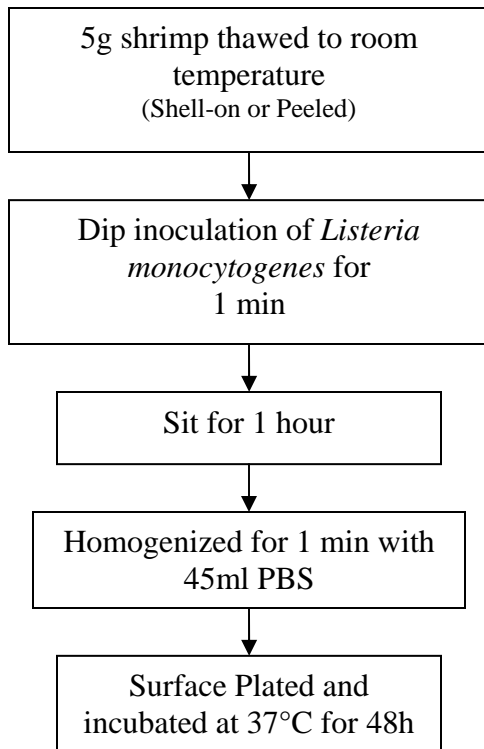
## APPENDIX 6

### PROCESS FLOW FOR CPC TREATMENT AGAINST *L. MONOCYTOGENES* ON THE SURFACE OF DOMESTIC AND IMPORTED SHRIMP STORED AT 4°C FOR 24H



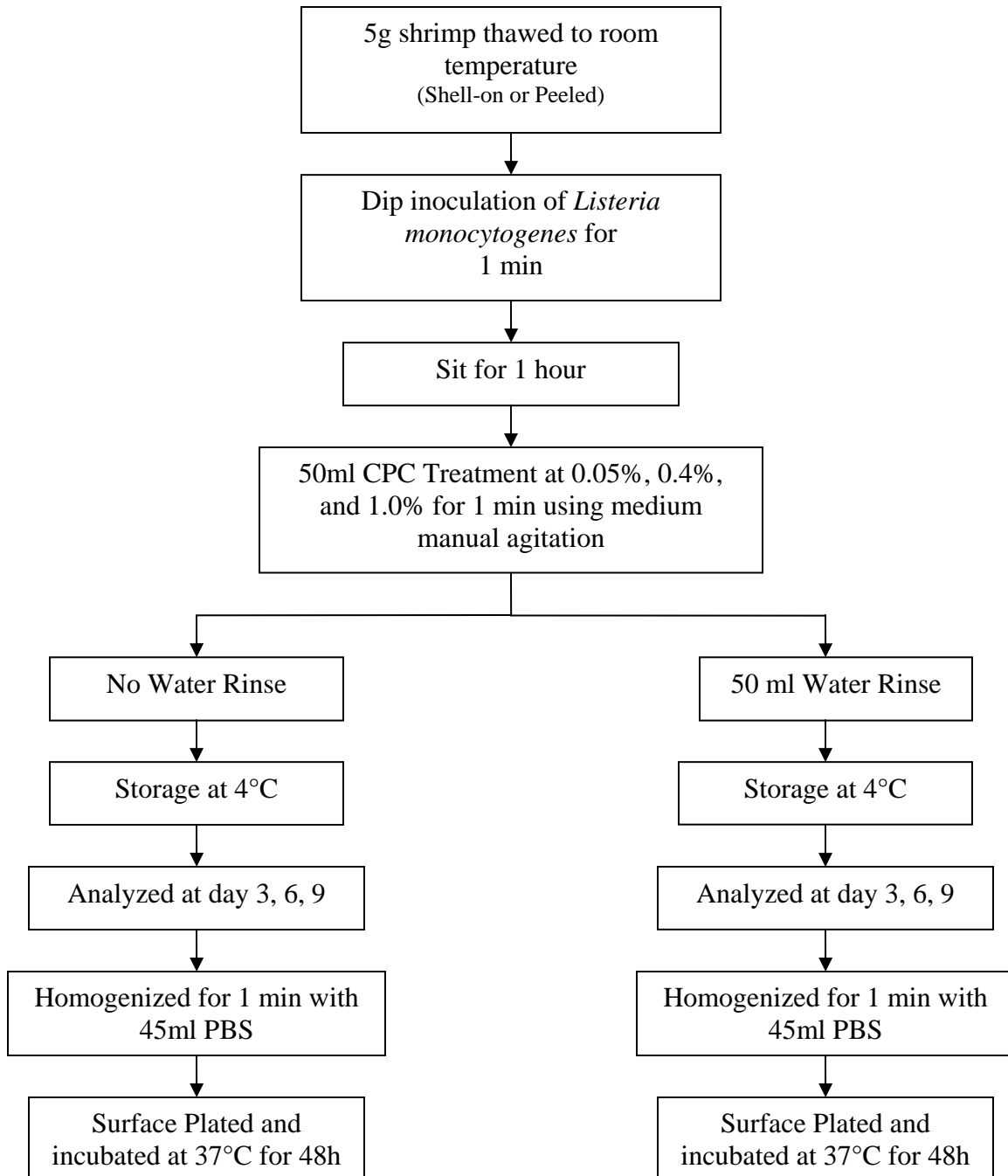
## APPENDIX 7

### PROCESS FLOW FOR ATTACHMENT OF *L. MONOCYTOGENES* ONTO THE SURFACE OF RETAIL (IMPORTED) SHRIMP



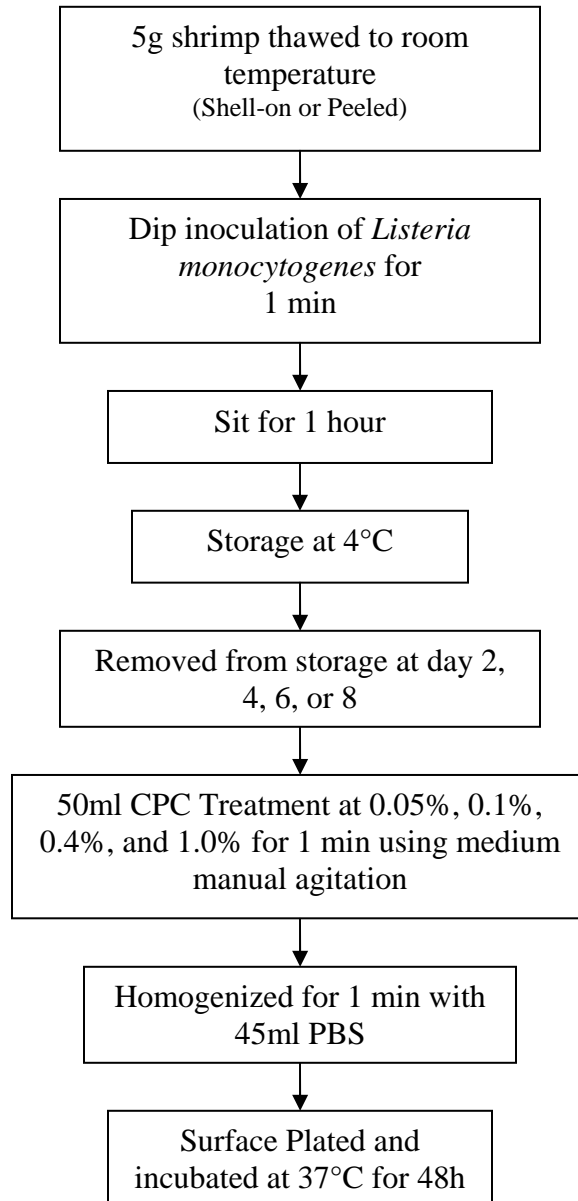
## APPENDIX 8

### PROCESS FLOW FOR CPC TREATMENT AGAINST *L. MONOCYTOGENES* ON THE SURFACE OF DOMESTIC SHRIMP STORED AT 4°C FOR 9 DAYS



## APPENDIX 9

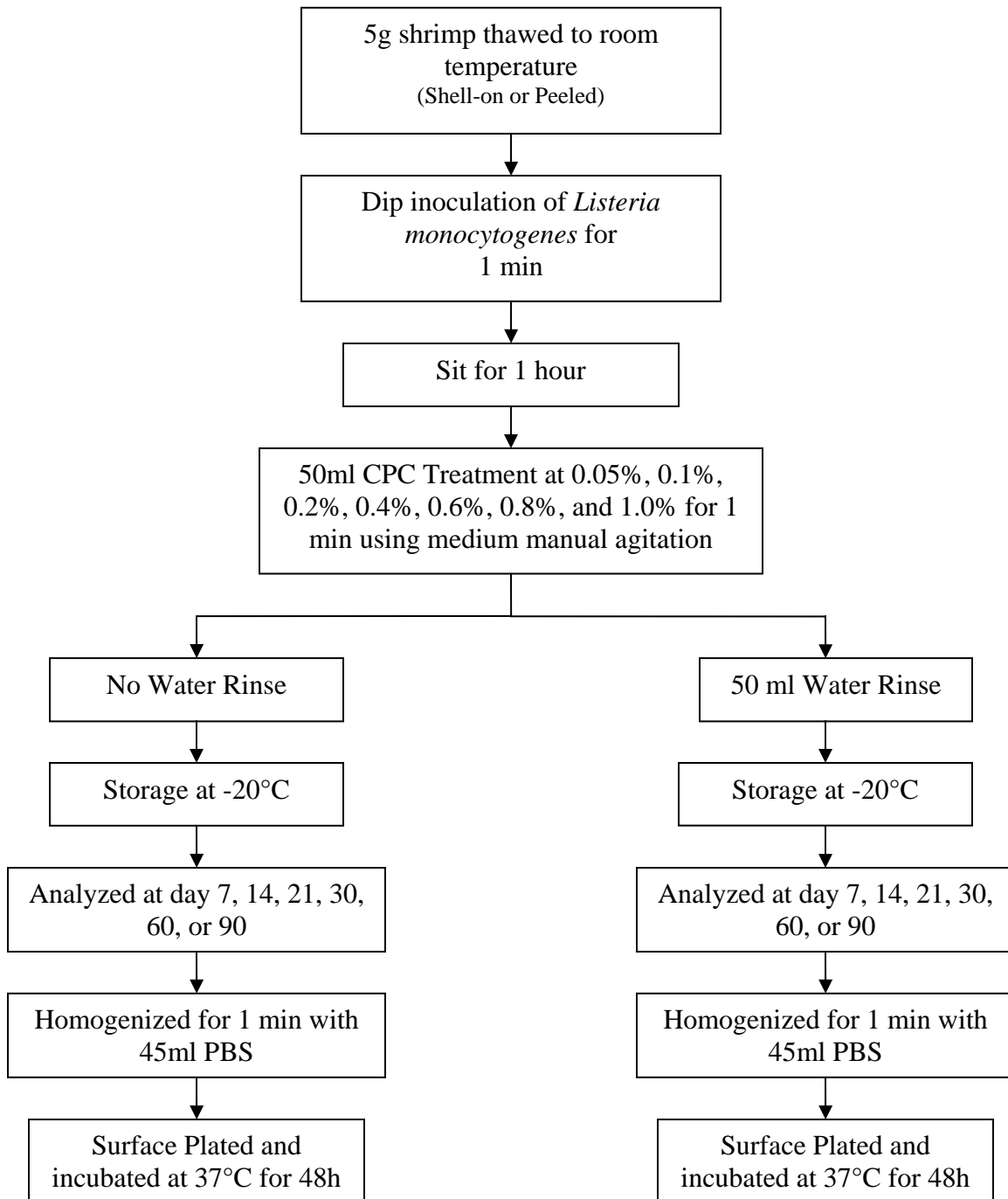
### PROCESS FLOW FOR CPC TREATMENT AGAINST *L. MONOCYTOGENES* BIOFILM ON THE SURFACE OF DOMESTIC SHRIMP STORED AT 4°C FOR 8 DAYS





## APPENDIX 10

### PROCESS FLOW FOR CPC TREATMENT AGAINST *L. MONOCYTOGENES* ON THE SURFACE OF DOMESTIC SHRIMP STORED AT -20°C FOR 90 DAYS



## **VITA**

Tracie Michelle Dupard was born on January 18, 1979, in Belleville, Illinois. She resided in New Orleans, Louisiana, where she received her high school and undergraduate education. In May 2001, she received her Bachelor of Science degree in environmental chemistry with dual concentration in biology and mathematics from Xavier University of Louisiana. She began pursuing a graduate degree at Louisiana State University and Agricultural and Mechanical College in the Department of Food Science in August 2003. After graduation, she will begin working at Nestle Prepared Foods Company located in Solon, Ohio.